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CONTENTS.

PAGE.
Proceedings ..... (33)-(40)

PAPERS.

On the Experimental Determination of the Hydrothermal Value of a Bomb Calorimeter. By H. W. Wiley and W. D. Bigelow 439
A Recalculation of Wein's Table of Starch Equivalent to Copper Found, Based on the Factor 0.92. By William H. Krug 452
The Proteids of Lupin Seeds. By Thomas B. Osborne and George F. Campbell 454
Effect of Minute Quantities of Acid on the Solubility of Globulin in Salt Solutions. By Thomas B. Osborne and George F. Campbell 482
The Proteids of the Sunflower Seed. By Thomas B. Osborne and George F. Campbell 487
The Proteids of the Cow Pea. By Thomas B. Osborne and George F. Campbell 494
On the Manufacture of Dynamite. By G. E. Barton 500
Proteid of the White Podded Adzuki Bean. By Thomas B. Osborne and George F. Campbell 509
Note: The Electrolytic Determination of Cadmium 513
Books Received 513

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A Modification of the Permanganate Method for the Determination of Iron. By Hamilton P. Cady and Alfred P. Ruediger.

The Exact Dilution of Liquid Reagents by Formula. By Chas. D. Howard.

On the Ferrocyanides of Zinc and Manganese. By Edmund H. Miller and J. A. Mathews.

The Ferrocyanides of Zinc and Manganese. By G. E. Stone and D. A. Van Ingen.

The Value of a Bacteriological Examination of Water from a Sanitary Point of View. By E. K. Dunham.

The Commercial Preparation of Nitronaphtalins. By Wm. H. Krug and J. E. Blomén.

Halides and Perhalides of Pyridine. By P. F. Trowbridge and O. C. Diehl.

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AMERICAN CHEMICAL SOCIETY.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE U. S. DEPARTMENT OF AGRICULTURE, NO. 27.]

ON THE EXPERIMENTAL DETERMINATION OF THE HYDROTHERMAL VALUE OF A BOMB CALORIMETER.

BY H. W. WILEY AND W. D. BIGELOW.

Received March 29, 1897.

FOUR methods of determining the water equivalent of a bomb calorimeter and its accessories may be employed.

In the first place, a definite weight of a body whose thermal value is established may be burned in the system in the usual way, and the increase of temperature of the ambient water, the weight of which is also known, be determined. The difficulty attending this method is, in the first place, that the heat of combustion of the body employed rests upon experimental data, which themselves may be misleading.

In the second place, without a tedious and accurate chemical examination the experimenter cannot be assured of the purity of his materials.

This method may be varied by the successive combustions of double or triple the weight of the same body, and thus a factor be secured from which the water value of the metal parts of the system can be accurately calculated.

The second method of operation is one which is employed by Berthelot and Vieille. It consists in measuring the heat disengaged in mixing exact weights of water and sulphuric acid. The operation is carried on first in an ordinary calorimeter without the bomb, and afterwards the mixture is made in the

interior of the bomb placed in position. The experiment being made upon absolute or relative weights permits of a comparison of the quantities of heat produced in the two conditions. This method of procedure, exact as it is in theory, is somewhat difficult in practice, because of the slowness with which the thermal equilibrium is established between two masses of liquid separated by a metallic wall.

The third method of estimating the hydrothermal value of the calorimeter system consists in calculating it directly from the specific heats of the various materials composing it.[1] This is by far the most convenient method of proceeding, and where the exact weights of the different constituents are known it leaves little to be desired. The specific heats of all the various metals composing the calorimetric system have been very carefully determined. It is only in the case of alloys of rather uncertain composition that errors may result. Most of the alloys, however, which are employed have a definite composition, or nearly so, and since the specific heats of metals are so low, the slight variations from the standard which may occur do not produce any appreciable errors. In all cases the hydrothermal value of the system should be calculated from the weights of its various components, and the manufacturers should always be asked to furnish the exact weights of each metal or alloy in the apparatus.

The fourth method of determining the hydrothermal value of the system is the direct experimental one of mixtures, the same in principle as the processes which have been used for the determination of the specific heats of various bodies. In the method which is to be described the steps will be set forth which are necessary in the determination of the hydrothermal value by the method of mixtures when the difference in temperature between the two bodies of water is not very pronounced. In all the methods of mixture which have heretofore been employed a relatively small volume of water at a relatively high temperature has been mixed with a large volume at ordinary temperature. In order to secure this mixture it has been customary to have the added water contained in a platinum capsule and placed as quickly as possible in the water in the calorimeter which has been kept at a definite temperature. After thorough mixing the in-

[1] Principles and Practice of Agricultural Analysis, 3, 573.

crease of temperature furnishes the basis of the calculations. In this case it is necessary to put the containing platinum capsule also in the mixture, and its hydrothermal value must be previously calculated by the usual factors. Thermometers which extend over large intervals of degrees are necessarily less exact and less easily read than those in which the scales mark only a few degrees. We therefore determined to attempt to ascertain the hydrothermal value of the calorimetric system by measuring the changes of temperature by means of the Beckmann thermometer employed for determining molecular weights. The scales of these thermometers usually extend over a little more than 5°, and are divided so as to read to hundredths, and, by means of a cathetometer, to thousandths of a degree. By using two of these thermometers it is possible to work with masses which have a difference in temperature of a little over 10°. Before the thermometers are used they must be calibrated against each other in order to be certain that they register exactly the same differences of temperature as marked upon the scales. This having been accomplished, the two thermometers are placed in water at room temperature, and when equilibrium has been perfectly established the two scales are read. The thermometers are set in such a way that when this comparison is made the column of mercury in one shall be near the top of the scale, and in the other one near the zero point. The difference in readings in the two thermometers is thus directly established, and this having been done, the two can be used as a single thermometer.

Following are the analytical data obtained in ascertaining by direct mixture the hydrothermal value of a bomb calorimeter made for this division by O. S. Blakeslee, of Middletown, Conn.:

Thermometers.—The two thermometers employed were made by F. O. R. Goetze, of Jena glass. The dimensions of the thermometers are as follows: No. 1 (Tl), distance from zero to bottom of bulb, twenty-five centimeters; length of scale, twenty-five centimeters; range of scale, zero to 5.6°. No. 2 (Ts), distance from zero to bottom of bulb, 17.5 centimeters; length of scale, twenty-two centimeters; range of scale, zero to 5.7°.

Immersed in water, at a temperature of 17.15°, the readings of the two thermometers were as follows:

No. 1, 0.030°; No. 2, 4.540°; difference, 4.510°.

Method of Using the Thermometers.—In order to secure as wide a range of temperature as possible with the two thermometers set as above, No. 2 was used for fixing the initial temperature of the water in contact with the bomb, and No. 1 for fixing the initial temperature of the water added to the bomb system. By this method of use a difference in initial temperatures of approximately 9° was easily obtained.

Method of Operation.—It is useless to attempt to determine the hydrothermal value of the bomb system immersed in air alone. The excessive slowness with which changes of temperature take place between polished metal and air is sufficient to prevent any accurate results from being obtained in an experimental way. Even after standing for several hours in air, a piece of polished metal may have a temperature which differs from that of the ambient air by as much as one degree or more. It is therefore necessary, at the very commencement, to add to the bomb system a sufficient quantity of water to secure without any great delay a uniform temperature in all its parts. In the apparatus employed the vessel containing the bomb holds approximately 1700 cc. of water when the bomb is in place. It is important that the range of temperature on mixing be as great as possible, as very small differences in temperature make a marked difference in the computations. Theoretically to get the best results the water which is added to the bomb at a higher temperature should be equivalent in weight to the water surrounding the bomb before mixture plus the hydrothermal equivalent of the calorimeter. The temperature of the bomb system, therefore, on mixing will rise to the same degree that the temperature of the added water falls. If, however, this arrangement should be followed, the quantity of water in contact with the bomb would not be sufficient to secure promptly the equilibrium of temperature between the water and the metallic parts of the calorimeter. To avoid any confusion of ideas, it may be stated that in the following experiments the added water was always at the higher temperature and the bomb system at the lower. After a large number of trials it was found that the best results, in the case under consideration, were secured by adding about 1000 cc. of water to the bomb in order to fix its initial temperature, and about 700 cc. at a higher temperature at the time of the determination. It was found

that with 1000 cc. of water only a portion of the upper part of the bomb remained uncovered. Theoretically, the best adaptation of the system would be such that a sufficient quantity of water might be introduced to cover the whole of the bomb and the containing vessel still be large enough to hold the rest of the water added at the time of the determination. Practically, the small portion of the metal covering of the bomb which remained uncovered by the water in the experiments indicated below, by reason of its high conductivity, speedily acquired the temperature of the mixture.

Manipulation.—It is advisable to select a day for the experiments which is cloudy, and in which the changes in temperature are very slight. We were fortunate, after all the preliminary work necessary to the determination had been done, to secure such a day for the actual fixing of the hydrothermal value of the system. The changes in temperature during the day in which the experiments were made, *viz.*, March 9, 1897, were very slight, and in the room in which the experiments were carried on the whole change during the working day did not exceed 3°.

It is convenient to have the thermometer so set that the initial temperature of the bomb system is slightly below the temperature of the room. In this case the initial temperature of the added water is considerably above the room temperature, and hence the rate of cooling is a more important factor in this instance than in the other. The first point to be determined is the rate and direction of change of temperature of the water and the whole calorimetric system. The stirrer of the apparatus having been set in motion, the whole is allowed to stand for about half an hour. The reading of the thermometer immersed in the water is then commenced, and continued for about ten minutes, the thermometer being read at each minute during this time. These successive readings fix the rate of change of temperature. At the moment the readings cease the exact time is noted, in order that the whole amount of change before the mixing takes place may be computed. The next point to be determined is the rate of change of temperature in the water which is to be added. This is determined in exactly the same way, but, as a rule, five observations are sufficient to fix this point. At the moment of ceasing the readings, the time

is also noted. The rates of change having been thus fixed, the thermometer is removed from the calorimeter system, and a funnel, which has been immersed in the water to be added, and is therefore at exactly the same temperature, and which has been used as the stirrer for this water, is inserted in the place of the thermometer, and the water poured into the calorimeter. The beaker and appurtenances having been previously weighed, a reweighing of the empty beaker permits of the exact determination of the weight of the water added. A large balance, capable of carrying heavy weights on each arm, and yet reading accurately to 10 milligrams, is necessary in weighing these large bodies. In order to secure as even a temperature as possible for the added water, the following device is employed: The water is contained in a lip beaker. This beaker is set in a tin can, which is insulated on the outside by a non-conducting covering of felt or cotton. The beaker rests upon three corks in the bottom of the can, and is separated from the sides by four corks inserted in such a way as to hold it firmly in place. The insulating covering is pushed down at the top between the tin can and the beaker, so that the air enclosed in the interior space is prevented from circulation with the exterior air. By this device the added water, which in these experiments was always several degrees higher in temperature than the ambient air, was protected as much as possible from rapid cooling. The time which is consumed in pouring the water, replacing the thermometer, and adjusting other parts of the apparatus, is noted, and from these data the initial temperatures of the bomb system and of the added water are calculated for the moments in which the last of the water is added. The thermometer at first indicates a rapid rise of temperature, which is observed through a reading glass, until it reaches its maximum. As a rule, it will be found that the added water causes the thermometer to rise to a slightly higher point than is indicated by the final equilibrium of temperature due to the fact that the mixture of the water affects the mercury in the thermometer with a slightly greater ease than it secures an equilibrium in the metal system. The difference, however, between the maximum reading of the thermometer in any case, and the final equilibrium is never more than a few thousandths. The temperature of the mixture is now read

as before, at intervals of a minute, until two successive readings are practically identical. The readings are then continued for a few minutes until the temperature begins to fall, and the rate of fall is established. The correct temperature of the mixture is determined by taking the mean of the two readings which are nearest together after the slight fall from the maximum temperature, produced on the addition of the water, and correcting this reading as described below. From the data thus obtained the hydrothermal value of the system is calculated. As an illustration of the methods of calculation, the following data of four determinations made on the 9th of March, 1897, are given:

RECORD OF DETERMINATIONS, MARCH 9, 1897.

Weight of water in contact with the bomb, 997.1 grams.
Commencement of readings, 9 o'clock, 58 minutes.
Readings of thermometer (Ts) at intervals of one minute, 1.471°, 1.468°, 1.468°, 1.468°, 1.467°, 1.465°, 1.462°, 1.460°, 1.460°, 1.460°.
Ending of readings, 10 o'clock, 7 minutes.
Corrected temperature of water added, 5.360°, thermometer (Tl).
Commencement of reading of water added, 10 o'clock, 8 minutes.
Readings at intervals of one minute, 5.594°, 5.560°, 5.525°, 5.485°, 5.450°, 5.420°.
End of reading, 10 o'clock, 13 minutes.
Time of pouring, etc., 2 minutes.
Reading of thermometer after mixture (Ts), 4.285°.
Other readings not entered.

Calculation.—The readings show that the temperature of the calorimetric system at the time of mixture was the same as that of the external air, so that no correction for changing temperature is to be applied. The temperatures of the added water show a change at the rate of 0.030° per minute. The time elapsed from the last reading until the pouring was complete was two minutes; hence the initial temperature of the added water was (Tl) 5.360°.

The weight of the added water was 710 grams. We then have the following data:

SUMMARY OF DATA.

Initial temperature of bomb and water (Ts)	1.460°
Initial temperature of added water (Tl) 5.360°, (Ts)	9.870°
Weight of added water	710 grams
Final temperature of mixture (Ts)	4.285°
Rise of temperature of calorimeter	2.825°
Fall of temperature of added water	5.585°

Then $710 \times 5.585 = x \times 2.825$ in grams of water.

Whence $x = 1403.7$, and $1403.7 - 997.1 = 406.6$ = hydrothermal value of the calorimetric apparatus in grams of water.

The method of correcting the reading of the thermometer for the influence of the environment after mixture is given is the next determination.

SECOND DETERMINATION.

Weight of water in contact with the bomb, 1034.2 grams.

Commencement of reading, 10 o'clock, 45 minutes.

Readings of thermometer at intervals of one minute, (Ts), 0.608°, 0.610°, 0.613°, 0.618°, 0.620°, 0.623°, 0.626°, 0.629°, 0.632°, 0.635°, 0.638°.

Ending of readings, 10 o'clock, 55 minutes.

First temperature of added water (T1), 5.585°.

Commencement of readings, 10 o'clock, 58 minutes.

Readings at intervals of one minute, 5.550°, 5.505°, 5.465°, 5.420°, 5.375° (T1).

End of readings, 11 o'clock, 3 minutes.

Time of pouring, 2 minutes.

The total time elapsed from the last reading of Ts, 0.638°, in the calorimeter until the pouring was complete, was ten minutes. The temperature of the calorimeter system was increasing at the rate of 0.003° per minute. The total increase during this time was therefore 0.030°, and the initial temperature of the bomb and water at the moment the pouring was complete was therefore $0.638° + 0.030° = 0.668°$. In the two minutes intervening between the completion of the pouring and the maximum reading of the thermometer, the change in temperature due to the environment is to be calculated from the observations of change after mixture, allowing for a temporary excess due to the time required for the metallic parts to assume the temperature of the ambient water. The rate of cooling, as determined by the final series of observations, was 0.004° per minute. The maximum temperature was observed at the end of two minutes, and the equilibrium of temperature was secured at the end of the third minute, when the observed temperature was (Ts) 3.620°. The loss of temperature for the three minutes which had elapsed since the completion of the mixture was therefore 0.012°, and this is to be added to the observed stable temperature (Ts) 3.620°. The final temperature of the mixture was therefore (Ts) $3.620° + 0.012° = 3.632°$.

SUMMARY OF DATA.

Reading of thermometer after mixture (Ts), 3.625°.

Time, 2 minutes.

Readings at intervals of one minute (Ts), 3.620°, 3.620°, 3.618°, 3.614°, 3.610°.

Initial temperature of bomb and water (Ts)	0.668°
Initial temperature of added water (Tl), 5.285°; (Ts)	9.795°
Weight of added water	693.3 grams
Final temperature of mixture (Ts)	3.632°
Rise of temperature of calorimeter	2.964°
Fall of temperature of added water	6.163°

Then $6.163 \times 693.3 = x \times 2.964$.

Whence $x = 1441.6$, and $1441.6 - 1034.2 = 407.4$ = hydrothermal value of apparatus in grams of water.

THIRD DETERMINATION.

Weight of water in contact with the bomb, 1006.4 grams.

Commencement of reading, 11 o'clock, 50 minutes.

Readings of the thermometer (Ts) at intervals of one minute, 0.835°, 0.840°, 0.845°, 0.850°, 0.857°, 0.865°, 0.870°, 0.875°, 0.880°, 0.885°.

Ending of readings, 12 o'clock.

First temperature of added water (Tl), 5.585°.

Readings of thermometer (Tl) at intervals of one minute, 5.555°, 5.525°, 5.490°, 5.450°, 5.415°.

Ending of readings, 12 o'clock, 10 minutes.

Time of pouring, 2 minutes.

Reading of thermometer (Ts) after mixture, 3.914° (maximum).

Time, 1 minute.

Readings of thermometer (Ts) in mixture at intervals of one minute, 3.912°, 3.911°, 3.910°, 3.908°, 3.903°.

Interval from last reading of bomb until pouring was complete, 12 minutes.

Rate of change in temperature, 0.005° +.

Temperature of bomb, etc., at completion of pouring, 0.0885° + 60.

(Ts) = 0.945°.

Last reading of temperature of added water (Tl), 5.415°.

Rate of change per minute, 0.035° −.

Time of pouring, 2 minutes.

Final temperature of added water at time pouring ceased (Tl), 5.415° − 0.070° = 5.345°.

The rate of change in the temperature of the mixture was 0.003° per minute.

Time from end of pouring to concordant reading of the thermometer, 3 minutes.

Correction to be applied to concordant reading, 3.911° (Ts), $0.003 \times 3 =$ 0.009.

Corrected reading of temperature of mixture 3.911°+0.009°=3.920° (Ts).

SUMMARY OF DATA.

Initial temperature of bomb and water (Ts)	0.945°
Initial temperature of added water (Tl), 5.345°; (Ts)	9.855°
Final temperature of mixture (Ts)	3.920°
Weight of water added	704 grams.
Rise of temperature of calorimeter	2.975°
Fall of temperature of added water	5.935°

Then $5.935 \times 704 = x \times 2.975$.

Whence $x = 1404.5$, and $1404.5 - 1006.4 = 398.1$=hydrothermal value of the calorimetric apparatus in grams of water.

FOURTH DETERMINATION.

Weight of water in contact with the bomb, 999.5 grams.

Commencement of readings, 2 o'clock, 8 minutes.

Reading of temperature of bomb (thermometer Ts) at intervals of one minute, 0.695°, 0.700°, 0.710°, 0.723°, 0.730°, 0.740°, 0.755°.

Ending of readings, 2 o'clock, 16 minutes.

First temperature of added water (Tl), 5.585°.

Commencement of readings, 2 o'clock, 17 minutes.

Readings of thermometer at intervals of one minute, 5.560°, 5.530°, 5.505°, 5.470°, 5.450.

Ending of readings, 2 o'clock, 23 minutes.

Time of pouring, 2 minutes.

Reading of thermometer (Ts) after end of pouring, 3.870°.

Readings of thermometer (Ts) in mixture at intervals of one minute, 3.875°, 3.875°, 3.875°, 3.875°.

SUMMARY OF DATA.

Initial temperature of bomb and water (Ts)	0.845°
Initial temperature of added water (Tl), 5.390°; (Ts)	9.900°
Final temperature of the mixture (Ts)	3.875°
Weight of water added	709.0 grams
Rise of temperature of calorimeter	3.030°
Fall of temperature of added water	6.025°

Then $709 \times 6.025 = x \times 3.03$.

Whence $x = 1409.8$, and $1409.8 - 999.5 = 410.3$ hydrothermal value of calorimetric apparatus in grams of water.

MEAN HYDROTHERMAL VALUE OF THE CALORIMETRIC SYSTEM.

From the data giving the hydrothermal values of the calorimeter, as determined in the foregoing experiments, we have the following table:

	Grams of water.
Hydrothermal value, first determination	406.6
" " second "	407.4
" " third "	398.1
" " fourth "	410.3
Mean hydrothermal value	405.6

COMPARISON OF HYDROTHERMAL VALUE OBTAINED EXPERIMENTALLY WITH THAT SECURED BY CALCULATION.

The specific heats of the various parts of the calorimetric system, as computed from the standard specific heats of the materials composing them, are given in the following table:

Material.	Specific heat.
Brass	0.093
Steel	0.1097
Platinum	0.0324
Copper	0.09245
Lead	0.0315
Oxygen	0.2389
Glass	0.190
Mercury	0.0332
Hard rubber	0.33125
Tin	0.0548
Antimony	0.0523

HYDROTHERMAL VALUE OF THE BOMB CALORIMETER.

The hydrothermal value of the bomb calorimeter is calculated from the following data:

Material.	Weight. Grams.	Specific heat.	Hydrothermal value. Grams of water.
Platinum	160.00 ×	0.0324 =	5.1840
Steel	3,228.10 ×	0.1097 =	354.1226
Lead	46.00 ×	0.0315 =	1.4490
Brass	65.39 ×	0.0930 =	6.0813
Brittania metal: tin	687.69 ×	0.0548 =	37.6854
Brittania metal: antimony	53.49 ×	0.0523 =	2.7974
Brittania metal: copper	22.92 ×	0.09245 =	2.1192
Mercury (estimated)	21.76 ×	0.0332 =	0.7224
Glass (estimated)	2.24 ×	0.1900 =	0.4256
Total hydrothermal value			410.5869

The two values, as obtained by direct determination and by calculation, are very near together. In the calculation above

given it should be borne in mind that it is difficult to determine the exact quantity of mercury in the thermometers without knowing the thickness of the sides of the bulb. It is also difficult to make the proper allowance for the glass part of the thermometer, inasmuch as it is not always immersed to exactly the same depth in the liquid, and further, the exact weight of the part immersed is not known, but can only be obtained approximately by calculation. Further, the tips of the wires, by which the firing of the bomb takes place, dip into the water, together with a portion of their caoutchouc covering. Further, the small piece of hard rubber by which one of the wires is insulated from the cover is not considered in the calculation. The hydrothermal value of these last-named bodies being slightly higher than metals, would have an appreciable influence upon the determination. The last thing to be mentioned in this connection is that while the specific heat of iron is pretty well established, it is known to vary slightly for the samples made in different localities and from different ores, and therefore the exact specific heat of steel, which composes the largest part of the calorimetric system, may be subject to slight variations. In view of this fact, it has been determined to use the mean of the two determinations as the correct expression for the hydrothermal value of the calorimeter in question, *viz.*, $(405.6 + 410.6) \div 2 = 408.1$.

CALCULATION BY THE FORMULA OF REGNAULT–PFAUNDLER.

The conditions of warming and cooling in the method of mixtures just described, are not precisely those which occur during the determination of the calorific power of a substance in the apparatus. The calculation of the influence of the ambient atmosphere upon the hydrothermal readings may, however, be conducted according to the formula of Regnault–Pfaundler.[1]

The calculation of the hydrothermal value in the case of the third determination given above, after correcting the end temperature according to the formula mentioned, is as follows:

Water value of the bomb, etc., x.

Weight of water added to the bomb, 1006.4 grams.

Preliminary thermometric readings, $t_1 = 0.835$, $t_2 = 0.840$, $t_3 = 0.845$, $t_4 = 0.850$, $t_5 = 0.857$, $t_6 = 0.865$, $t_7 = 0.870$, $t_8 = 0.875$, $t_9 = 0.880$, $t_{10} = 0.885$, $t_{n_1} = 0.945$. $(n_1 = 22)$.

[1] Principles and Practice of Agricultural Analysis, **3**, 572, 574.

Thermometric readings after pouring, $\theta_1 = 0.945$, $\theta_2 = 3.914$, $\theta_3 = 3.912$, $\theta_n = 3.911$. $(n = 4)$.

Final thermometric readings, $t'_1 = 3.911$, $t'_2 = 3.910$, $t'_3 = 3.908$, $t'_{n_2} = 3.903$.

From the formulas given above the following numerical values are computed:

$$v = \frac{t_{n_1} - t_1}{n_1 - 1} = 0.005.$$

$$v' = \frac{t'_{n_2} - t'_1}{n_2 - 1} = -0.003.$$

$$t = \frac{t_1 + t_2 + t_3 \cdots\cdots t_n}{n_1} = 0.891.$$

$$t' = \frac{t'_1 + t'_2 + t \cdots\cdots t'_{n_2}}{n_2} = 3.908.$$

$$\sum_1^{n-1} \theta r = \theta_1 + \theta_2 + \theta_3 + \frac{\theta_2 - \theta_1}{9} = 9.108.$$

Substituting these values in the Regnault–Pfaundler formula, the correction for the influence of the external air is

$$\Sigma \Delta t = \frac{0.005 - (-0.003)}{3.903 - 0.891}\left(9.10 + \frac{3.911 + 0.945}{2} - (4 \times 0.891)\right) - (3 \times 0.005) = 0.006,$$

which is added to the end temperature $(\theta_n = 3.911)$.

The computation is then made from the following data:

Corrected end temperature $(\theta_n + 0.006)$	3.917°
Beginning temperature (θ_1)	0.945°
Increase in temperature of bomb, etc	2.972°
Total calories	x 2.972°
Weight of added water	704.0 grams
Temperature of added water	9.855°
Fall of temperature of added water	5.938°

Then $5.938 \times 704 = x \times 2.972$. Whence $x = 1406.6$, and $1406.6 - 1006.4 = 400.2$ = water value of calorimeter.

Calculated directly by the methods first given, the hydrothermal value in the third determination is found to be 398.1. After correcting for the end temperature by the Regnault–Pfaundler formula, and applying the calculation the hydrothermal value is found to be 400.2. It is thus seen that the two methods give almost the same results. For all practical purposes, therefore, it is not necessary to undertake the complicated computations required by the Regnault–Pfaundler formula, but the data may be calculated directly by the methods first given.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE U. S. DEPARTMENT OF AGRICULTURE, No. 28.]

A RECALCULATION OF WEIN'S TABLE OF STARCH EQUIVALENT TO COPPER FOUND, BASED ON THE FACTOR 0.92.[1]

BY WILLIAM H. KRUG.

Received March 24, 1897.

IN Wein's "Tabellen zur quantitativen Bestimmung der Zuckerarten" a table is given on page 41, by means of which the starch or dextrin can be directly obtained from the copper found by converting the starch into dextrose and determining the latter with Allihn's solution. This table is based on the factor 0.90, which assumes that the formula of starch is $(C_6H_{10}O_5)_n$, and that it is all converted into dextrose. Nägeli determined the formula of starch to be $C_{36}H_{62}O_3$, and if this be correct the factor becomes 0.918. Ost, working with the Sacchse method decided upon the factor 0.925. In view of all these conflicting data Wiley recommends the factor 0.92, a mean between the two last cited, which will give the analyst fairly accurate results. This factor has been used in the recalculation of the table.

Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.
10..	5.6	30..	14.7	50..	23.8	70..	32.9	90..	42.2
11..	6.1	31..	15.2	51..	24.3	71..	33.4	91..	42.7
12..	6.5	32..	15.6	52..	24.7	72..	33.9	92..	43.1
13..	7.0	33..	16.1	53..	25.2	73..	34.3	93..	43.6
14..	7.5	34..	16.6	54..	25.7	74..	34.8	94..	44.1
15..	7.9	35..	17.0	55..	26.1	75..	35.1	95..	44.5
16..	8.3	36..	17.4	56..	26.5	76..	35.7	96..	45.0
17..	8.7	37..	17.8	57..	27.0	77..	36.2	97..	45.4
18..	9.2	38..	18.3	58..	27.4	78..	36.5	98..	45.9
19..	9.7	39..	18.8	59..	27.9	79..	37.1	99..	46.4
20..	10.1	40..	19.3	60..	28.3	80..	37.5	100..	46.8
21..	10.5	41..	19.7	61..	28.8	81..	38.0	101..	47.3
22..	11.0	42..	20.1	62..	29.3	82..	38.5	102..	47.7
23..	11.5	43..	20.6	63..	29.7	83..	38.9	103..	48.2
24..	12.0	44..	21.1	64..	30.2	84..	39.4	104..	48.7
25..	12.4	45..	21.5	65..	30.6	85..	39.9	105..	49.2
26..	12.9	46..	22.0	66..	31.1	86..	40.4	106..	49.7
27..	13.3	47..	22.4	67..	31.6	87..	40.8	107..	50.1
28..	13.8	48..	22.9	68..	32.0	88..	41.3	108..	50.6
29..	14.3	49..	23.4	69..	32.5	89..	41.8	109..	51.1

[1] Read at the meeting of the Washington Section, March 11, 1897.

Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.
110..	51.5	152..	71.3	194..	91.5	236..	112.0	278..	132.8
111..	52.0	153..	71.8	195..	92.0	237..	112.5	279..	133.4
112..	52.4	154..	72.3	196..	92.5	238..	113.0	280..	133.9
113..	52.9	155..	72.8	197..	92.9	239..	113.5	281..	134.4
114..	53.4	156..	73.2	198..	93.4	240..	114.0	282..	134.9
115..	53.9	157..	73.7	199..	93.8	241..	114.5	283..	135.4
116..	54.3	158..	74.2	200..	94.4	242..	115.0	284..	135.9
117..	54.7	159..	74.7	201..	94.9	243..	115.5	285..	136.4
118..	55.3	160..	75.2	202..	95.4	244..	115.9	286..	136.9
119..	55.8	161..	75.6	203..	95.9	245..	116.5	287..	137.4
120..	56.2	162..	76.1	204..	96.3	246..	116.9	288..	137.8
121..	56.7	163..	76.6	205..	96.9	247..	117.4	289..	138.5
122..	57.1	164..	77.1	206..	97.3	248..	117.8	290..	138.9
123..	57.6	165..	77.6	207..	97.8	249..	118.4	291..	139.5
124..	58.1	166..	78.0	208..	98.3	250..	118.9	292..	139.9
125..	58.5	167..	78.5	209..	98.8	251..	119.3	293..	140.5
126..	59.1	168..	79.0	210..	99.3	252..	119.9	294..	140.9
127..	59.5	169..	79.5	211..	99.7	253..	120.3	295..	141.5
128..	60.0	170..	79.9	212..	100.3	254..	120.9	296..	142.0
129..	60.4	171..	80.4	213..	100.7	255..	121.3	297..	142.5
130..	60.9	172..	80.9	214..	101.2	256..	121.8	298..	143.0
131..	61.4	173..	81.4	215..	101.8	257..	122.4	299..	143.5
132..	61.8	174..	81.9	216..	102.2	258..	122.8	300..	144.0
133..	62.3	175..	82.3	217..	102.7	259..	123.4	301..	144.5
134..	62.7	176..	82.8	218..	103.1	260..	123.8	302..	145.0
135..	63.3	177..	83.3	219..	103.7	261..	124.3	303..	145.5
136..	63.8	178..	83.8	220..	104.0	262..	124.8	304..	146.0
137..	64.2	179..	84.3	221..	104.6	263..	125.3	305..	146.6
138..	64.7	180..	84.7	222..	105.2	264..	125.9	306..	147.0
139..	65.1	181..	85.2	223..	105.6	265..	126.3	307..	147.6
140..	65.6	182..	85.6	224..	106.1	266..	126.8	308..	148.0
141..	66.1	183..	86.2	225..	106.6	267..	127.3	309..	148.6
142..	66.5	184..	86.7	226..	107.1	268..	127.8	310..	149.0
143..	67.1	185..	87.1	227..	107.5	269..	128.3	311..	149.6
144..	67.5	186..	87.6	228..	108.0	270..	128.8	312..	150.0
145..	68.0	187..	88.0	229..	108.5	271..	129.4	313..	150.6
146..	68.4	188..	88.6	230..	109.0	272..	129.8	314..	151.1
147..	68.9	189..	89.1	231..	109.5	273..	130.3	315..	151.6
148..	69.4	190..	89.5	232..	110.0	274..	130.8	316..	152.1
149..	69.9	191..	90.0	233..	110.5	275..	131.4	317..	152.6
150..	70.3	192..	90.5	234..	111.0	276..	131.8	318..	153.1
151..	70.8	193..	91.0	235..	111.5	277..	132.4	319..	153.6

Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.
320..	154.1	349..	169.0	378..	184.3	407..	199.6	436..	215.2
321..	154.7	350..	169.6	379..	184.7	408..	200.1	437..	215.7
322..	155.1	351..	170.1	380..	185.3	409..	200.7	438..	216.3
323..	155.7	352..	170.6	381..	185.8	410..	201.2	439..	216.8
324..	156.1	353..	171.1	382..	186.3	411..	201.8	440..	217.4
325..	156.7	354..	171.6	383..	186.9	412..	202.3	441..	217.9
326..	157.2	355..	172.1	384.	187.4	413..	202.8	442..	218.5
327..	157.7	356..	172.7	385..	188.0	414..	203.3	443..	219.1
328..	158.2	357..	173.2	386..	188.4	415..	203.9	444..	219.6
329..	158.7	358..	173.8	387..	189.0	416..	204.4	445..	220.2
330..	159.2	359..	174.2	388..	189.5	417..	205.0	446..	220.6
331..	159.8	360..	174.8	389..	190.0	418..	205.4	447..	221.2
332..	160.3	361..	175.4	390..	190.5	419..	206.0	448..	221.7
333..	160.8	362..	175.8	391..	191.1	420..	206.5	449..	222.3
334..	161.3	363..	176.4	392..	191.6	421..	207.1	450..	222.8
335..	161.8	364..	176.9	393..	192.1	422..	207.6	451..	223.4
336..	162.4	365..	177.5	394..	192.6	423..	208.2	452..	223.9
337..	162.8	366..	177.9	395..	193.2	424..	208.7	453..	224.4
338..	163.4	367..	178.5	396..	193.8	425..	209.3	454..	225.0
339..	163.9	368..	179.0	397..	194.3	426..	209.8	455..	225.6
340..	164.4	369..	179.5	398..	194.8	427..	210.3	456..	226.0
341..	165.0	370..	180.0	399..	195.3	428..	210.9	457..	226.6
342..	165.4	371..	180.6	400..	195.9	429..	211.4	458..	227.1
343..	166.0	372..	181.1	401..	196.4	430..	212.0	459..	227.7
344..	166.4	373..	181.6	402..	197.0	431..	212.5	460..	228.3
345..	167.0	374..	182.1	403..	197.4	432..	213.1	461..	228.8
346..	167.5	375..	182.7	404..	197.9	433..	213.6	462..	229.4
347..	168.0	376..	183.2	405..	198.5	434..	214.2	463..	230.0
348..	168.5	377..	183.7	406..	199.1	435..	214.7		

THE PROTEIDS OF LUPIN SEEDS.[1]

By Thomas B. Osborne and George F. Campbell.

Received April 12, 1897.

THE lupin is a leguminous plant little known in this country except as a garden ornament. The yellow lupin (*Lupinus luteus*) and the blue lupin (*Lupinus angustifolius*), both native to Mediterranean regions, have long been cultivated in Europe because of their ability to grow luxuriantly on sandy or gravelly soils, and by their help large areas of poor, "worn out" land have been reclaimed and made agriculturally profitable, as these plants furnish abundant fodder and by the decay of their deeply penetrating roots, and especially when plowed under

[1] From the Report of the Connecticut Agricultural Experiment Station for 1896.

green, they rapidly impregnate the soil with humus and render it productive for other crops.

Ritthausen[1] first studied and described under the name *conglutin*, the characteristic proteid of the lupin seed. He found that the yellow and the blue lupin both yielded conglutin scarcely distinguishable in properties and only differing in composition as respects sulphur, of which his preparations from the yellow lupin contained one per cent. and those from the blue lupin but one-half per cent. Ritthausen also analyzed preparations which, from their composition, he concluded to be legumin. He stated that both legumin and conglutin are extracted from lupin seeds by salt solutions as well as by dilute alkali, and that the two proteids can be separated from each other by dissolving in alkali, then precipitating with an acid and finally treating with salt solution. On neutralizing these alkali solutions conglutin retains, while legumin loses its solubility in saline solutions.

By extracting with five per cent. sodium chloride brine Ritthausen obtained two preparations from yellow lupin having the composition given under 1 and 2; by dissolving 2 in potash water and reprecipitating, 3 was prepared; the blue lupin by extracting with salt solution yielded 4.

CONGLUTIN, RITTHAUSEN.

	Yellow Lupin.			Blue Lupin.
	1	2	3	4
Carbon	50.40	50.58	50.26	50.39
Hydrogen	7.00	7.06	6.89	6.94
Nitrogen	18.34	18.04	18.28	18.22
Sulphur }	24.26	24.32	1.01	0.49
Oxygen }			23.56	23.96
	100.00	100.00	100.00	100.00

The proteid rendered insoluble in salt solution by previous precipitation from an alkaline liquid, by acetic acid, he found to have the following composition, these figures being the average of five quite closely agreeing analyses:

LEGUMIN, RITTHAUSEN.

Carbon	51.36
Hydrogen	6.97
Nitrogen	17.50
Sulphur	0.59
Oxygen	23.58
	100.00

[1] *J. prakt. Chem.*, 103, 78: *Ibid*, New Series, 24, 222, and 26, 422, and *Die Eiweisskörper*, etc., *Bonn*, 1872.

Palladin[1] has recently described the properties of "vitellin" contained in seeds of the yellow lupin. In many details the results of his work do not agree with ours, but as he admits that the "vitellin" which he examined was always somewhat impure, it is unnecessary to review his statements here.

YELLOW LUPIN.

We find that seeds of the yellow lupin contain a small quantity of proteid that is soluble in pure water, a large quantity soluble in salt solutions, a small amount soluble in potash water, and a little nitrogenous matter, presumably proteid, which cannot be extracted by these solvents.

To determine the proportions of these proteids, very finely ground yellow lupin meal was first completely extracted with warm alcohol of 0.868 sp. gr. in order to remove, as far as possible, the amides and alkaloids which occur in considerable quantities in this seed. The air-dried meal before exhaustion with alcohol contained 8.16 per cent. of nitrogen. Alcohol removed eleven per cent. of the meal and gave a residue containing 8.49 per cent. of nitrogen, showing that six-tenths per cent. of nitrogen calculated on the original meal had been removed. The original meal accordingly contained 7.56 per cent. of nitrogen in the form of compounds insoluble in alcohol. As the seed reacted strongly acid towards litmus it is possible that a considerable quantity of alkaloids was not removed by the alcohol, but it was not desirable to add any alkali in this extraction for fear of affecting the proteids.

One thousand grams of the alcohol-exhausted and thoroughly air-dried meal were treated with successive quantities of ten per cent. sodium chloride solution until saturation of the filtered extract with ammonium sulphate showed that no more proteid was removed. The united extracts were filtered clear and saturated with ammonium sulphate; the precipitated proteids were dissolved in brine and the solution filtered and dialyzed for several days. The abundant precipitate thus produced was filtered out, washed by decantation with water and with alcohol and dried over sulphuric acid. The substance thus obtained weighed 279 grams.

The solution filtered from this globulin was saturated with

[1] *Ztschr. fur. Biol.*, 31, 191.

ammonium sulphate, the precipitate produced was dissolved in water and the solution filtered and dialyzed until no more globulin separated. By this second dialysis 15.2 grams of globulin were secured. The solution filtered from this second portion of globulin was concentrated by dialyzing in alcohol and the proteid completely thrown down by adding absolute alcohol. The precipitate which resulted was then dehydrated with absolute alcohol and dried over sulphuric acid. Only 4.20 grams of substance were thus obtained, indicating the presence of very little proteid soluble in water.

The residue of meal was washed with water, which took up no proteid, and exhausted by successive applications of two-tenths per cent. potash water. The alkaline extracts were filtered clear and treated with dilute acetic acid. No precipitate resulted until a decided excess of acid had been added. The proteid thus separated was washed with water and with alcohol, dried over sulphuric acid and weighed 60.53 grams.

The meal residue was next washed with water and with alcohol, dehydrated with absolute alcohol and thoroughly air-dried. It weighed 192.0 grams, showing the lupin seed to contain over eighty per cent. of substance soluble in salt solution and very dilute alkali, an amount not approached by any other seed which we have examined.

The nitrogen was determined in the various products and the results are summarized in the following table:

AMOUNT OF PROTEID EXTRACTED FROM YELLOW LUPIN BY VARIOUS SOLVENTS.

		Grams.
Extracted residue contained 1.51 per cent. N. or		2.90
Proteid extracted by alkali,	60.53 grams, contained 16.43 per cent. N. or	9.95
Proteid extracted by salt solution	279.0 grams, contained 17.86 per cent. N. or	49.83
	15.2 grams, contained 18.09 per cent. N. or	2.75
Proteid extracted by water,	4.2 grams, contained 16.55 per cent. N. or	0.69
	Total proteid, 358.93 grams.	
Total nitrogen accounted for		66.12
1,000 grams of meal contained 8.49 per cent. N. or		84.90

We thus have 77.88 per cent. of the nitrogen accounted for.

We will next consider in order:

I. Proteids soluble in pure water.

II. Proteids insoluble in water, but soluble in sodium chloride solution.

III. Proteids insoluble in water and in salt solution, but soluble in dilute potash water.

I. *Proteids Soluble in Pure Water.*

As just indicated, water-soluble proteids occur in yellow lupin seeds in very small amount. Much the largest quantity obtained in any of the numerous extractions made was that already described, which formed 0.42 per cent. of the alcohol-extracted meal or 0.37 per cent. of the original meal. On treating this substance with water, a very considerable part was found to be coagulated. Since proteoses are not supposed to be rendered insoluble by prolonged treatment with alcohol, this insoluble matter was probably coagulated albumin or globulin, or possibly a mixture of both. The aqueous solution filtered from this insoluble proteid yielded a very small flocculent coagulum at 59°. The solution filtered after heating to 65°, became turbid at 67°, and flocks in minute amount appeared at 69°, which steadily increased until at 85° a very considerable coagulum had formed.

In another extraction by means of water, prolonged dialysis caused the separation of a small quantity of globulin which, when dissolved in salt solution, yielded a flocculent coagulum at 59° and but traces above 65°.

The solution from which most of this globulin had been removed by dialysis, gave a slight coagulum at 59°, and after heating to 65° the filtrate became turbid at 66°, and at 84° again yielded a small flocculent coagulum.

It is probable, therefore, that the substance coagulating at 59° is a globulin soluble in extremely dilute salt solutions which it is impossible to separate completely by dialysis and that the proteid coagulating at the higher temperature is an albumin.

Owing to the exceedingly small quantity of these proteids nothing further was learned respecting them.

The solution from which the coagulated proteid had been removed by heating gave a strong rose-red reaction with the

biuret test, but no precipitate in the cold with nitric acid even after adding salt.

The yellow lupin seed accordingly contains a very small amount of albumin and a small quantity of proteose.

Neumeister[1] states that he found peptone in lupin seeds in large amount.

In order to test the seeds of the yellow lupin for peptones, 1,000 grams of freshly ground meal were treated with three liters of distilled water, and after agitating therewith for an hour the extract was strained through fine bolting-cloth and the residue pressed out in a powerful screw press. Two liters of extract rich in dissolved substances was obtained, which was immediately saturated with ammonium sulphate and filtered. Lest peptones might be formed during the operation of extraction, the process up to this point was carried forward as rapidly as possible so that not over three hours elapsed before the solution, saturated with ammonium sulphate, was filtered. In order to be sure that the solution had been thoroughly saturated, a quantity of crystals of this salt were suspended in it over night, but no more proteid separated. Neumeister has stated that saturating seed extracts while hot and when made alternately acid and alkaline in reaction, is unnecessary. We, however, heated the solution to boiling, added ammonium sulphate as long as it dissolved, concentrated somewhat and allowed to cool. Much ammonium sulphate separated, but no noticeable quantity of proteose. After filtering off the separated sulphate, the solution was heated to boiling and concentrated until sulphate began again to crystallize out. Ammonia was next added to distinct alkaline reaction, and after heating a short time the solution was again cooled, filtered from deposited sulphate, and the filtrate concentrated until more sulphate separated. Acetic acid was added to acid reaction, the heating continued for a time, and the whole again cooled. After filtering out the separated crystals the solution measured 350 cc. The solution was then nearly neutralized with ammonia, leaving its reaction slightly acid, and after adding an equal volume of water a freshly prepared solution of tannic acid was gradually added so long as a precipitate was produced in a small portion of the filtered liquid. A bulky reddish precipitate

[1] *Ztschr. für. Biol., n. f.*, 12, 461.

formed which, after standing twenty-four hours, was removed from the filter and treated with a slight excess of hot concentrated solution of baryta. After standing a short time the warm solution was filtered, and as it was very strongly alkaline from free ammonia, one-half was neutralized with sulphuric acid, thereby removing the excess of baryta. Neutral lead acetate was then added and the solution filtered. The most carefully applied biuret test did not show the least trace of peptone in this solution. The remainder was then evaporated nearly to dryness and about two cc. of syrup obtained which, if it had all been peptone, would hardly be considered a large amount. No peptone reaction whatever could be obtained with this syrup.

The other half of the solution was treated exactly as Neumeister directed—that is, neutral lead acetate was added without neutralizing the ammonia, and, after filtering, the biuret reaction was applied, but with no indication of peptone.

Since writing the above, S. Frankfurt[1] has stated that no peptone is present in seeds of the lupin, and attributes Neumeister's results to his long treatment of the seeds with water. In a letter to Frankfurt, Neumeister stated that after swelling the seeds in water they were rubbed up and digested with water for twenty-four hours, and that the vessels containing the extracting seeds were exposed to the direct action of sunlight in summer. It is thus evident that the peptone found by Neumeister was formed during the extraction and was not an original constituent of the seed.

II. *Proteids Soluble in Saline Solutions.*

As just mentioned, a very small quantity of a globulin soluble in extremely dilute salt solutions, yielding a flocculent coagulum when its solution in ten per cent. sodium chloride is heated to 59°, was found in extracts of the yellow lupin seed. As but little of this proteid is present in this seed, no attempt was made to do more than note its presence. Owing to its ready solubility in very dilute salt solutions, this proteid dissolves when lupin meal is treated with water. Besides this little if any other globulin substance is extracted from lupin seeds by the dilute saline solution which results when the meal is treated with water. Large

[1] *Landw. Ver.-Stat.*, 47, 454.

quantities of globulin are, however, obtained by extraction with stronger salt solutions.

One hundred grams of meal yielded directly with salt solution an extract which was filtered clear, saturated with ammonium sulphate, the precipitate produced filtered out, dissolved in brine, and the solution filtered clear and dialyzed for eighteen hours. The globulin thus precipitated was filtered out, washed with water, alcohol, and ether, dried over sulphuric acid, and found to weigh twelve grams. The filtrate by further dialysis similarly yielded four and seven-tenths grams of globulin. These preparations, 1 and 2, were analyzed with the results given below. The residue, remaining after exhausting 100 grams of meal with water, on treating with salt solution gave an extract which, when filtered clear and dialyzed four days, yielded 20.36 grams of globulin, preparation 3, having the composition given in the following table. A large quantity of the globulin was prepared by extracting one kilogram of finely ground lupin meal with six liters of brine, filtering the resulting solution, saturating with ammonium sulphate, filtering out the precipitate produced, dissolving this in dilute salt solution, again precipitating by saturating with ammonium sulphate, redissolving the precipitate, filtering the solution so obtained and dialyzing for forty hours. The very large precipitate which separated was washed thoroughly by decantation with water, dilute alcohol, absolute alcohol and ether and then dried over sulphuric acid. In this way 112 grams of preparation 4 were secured having the composition given in the subjoined table. The filtrate from 4, after three days further dialysis, gave a second precipitate of globulin which was decidedly more viscid than 4. This by the usual treatment yielded 30 grams of preparation 5 with the composition given below.

Another portion of meal weighing one kilogram was several times extracted with water and the residue treated with successive applications of ten per cent. salt solution. The extract was filtered clear and dialyzed for forty hours. The globulin so precipitated was thoroughly washed with water by decantation, then with dilute alcohol and then with absolute alcohol until no more color was removed, and finally with ether and then dried over sulphuric acid; 120 grams of preparation 6 were thus

obtained, giving on analysis the figures stated below. The solution from which 6 had separated was saturated with ammonium sulphate, the precipitate dissolved in brine and the solution returned to the dialyzer. After some days the small precipitate which had separated was filtered out, washed and dried in the usual manner and gave five grams of preparation 7 having the composition given below.

In order to avoid as far as possible any contamination of the proteid with nitrogenous or other substances soluble in alcohol, a quantity of very fine ground lupin meal was exhausted in Squibb's percolator with a large quantity of strong alcohol, the process being continued until only a trace of solid matter remained after evaporating a considerable quantity of the alcoholic extract.

Two kilograms of this meal were then extracted as thoroughly as possible with brine, the solution filtered clear and saturated with ammonium sulphate. The proteids thus precipitated were dissolved in brine, the solution filtered perfectly clear and the globulin thrown down by dialyzing three days was filtered off, washed and dried in the manner already described. There was thus obtained 506 grams of globulin, preparation 8.

The solution filtered from 8 by longer dialysis yielded forty-five grams of preparation 9. These preparations had the composition given below.

LUPIN GLOBULIN.

	1.	2.	3.	4.	5.	6.	7.	8.	9.
Carbon	50.62	50.68	50.63	50.49	50.29	50.41	49.81	50.20	49.47
Hydrogen ..	6.94	6.95	7.00	6.77	6.89	6.85	6.79	6.75	6.77
Nitrogen ...	17.45	17.89	18.05	17.90	17.88	18.01	17.79	17.86	18.07
Sulphur	0.77	0.80	0.88	0.88	1.25	0.87	1.48	0.98	1.49
Oxygen.....	24.22	23.68	23.44	23.96	23.69	23.86	24.13	24.21	24.20
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Ash.....	0.51	0.27	0.74	0.26	0.30	0.99	0.83	0.38	0.23

These figures agree quite closely except those for 5, 7, and 9 in which carbon is less and sulphur decidedly more than in the others. The fact that these three preparations separated on prolonged dialysis of the solutions which had yielded 4, 6, and 8 indicates the presence of two globulins of different composition.

It was therefore necessary to submit this substance to very thorough fractional precipitation in order to determine definitely its true composition. Accordingly, 100 grams of preparation 6

were dissolved in 800 cc. of five per cent. salt solution, filtered from a very small quantity of insoluble matter, and the perfectly clear solution was mixed with 800 cc. of water and cooled to 0°. A very large proportion of the dissolved proteid separated as a solid, coherent mass on the bottom of the beaker, from which the clear solution was completely decanted. This precipitate was marked *A*, the solution *B*. The precipitate *A* was dissolved in 100 cc. of ten per cent. brine, yielding readily a perfectly clear solution. This was mixed with 100 cc. of water at 20° but no precipitate resulted. Water was then added gradually until an abundant precipitate formed and the total volume equalled 500 cc. After settling, the clear solution was poured off from the precipitate and the latter washed thoroughly with water and alcohol and dried over sulphuric acid. In this way 46 grams of preparation 10 were obtained, having the following composition :

LUPIN GLOBULIN, 10.

Carbon	50.49
Hydrogen	6.77
Nitrogen	17.89
Sulphur	0.66
Oxygen	24.19
	100.00
Ash	0.51

The solution decanted from 10 was mixed with 600 cc. more water, which gave another precipitate that, after washing and drying, weighed ten and a half grams and had the composition given below.

LUPIN GLOBULIN, 11.

Carbon	50.18
Hydrogen	6.94
Nitrogen	17.93
Sulphur	0.82
Oxygen	24.13
	100.00
Ash	0.77

The solution *B* decanted from the first precipitate *A*, as described above, was diluted with an equal volume of water, cooled to 0°, and allowed to deposit the resulting precipitate. After washing and drying, preparation 12, weighing eight

grams, was obtained, which gave the following results on analysis :

LUPIN GLOBULIN, 12.

Carbon	50.08
Hydrogen	6.82
Nitrogen	18.26
Sulphur	1.30
Oxygen	23.54
	100.00
Ash	0.74

The solution from which 12 separated was saturated with ammonium sulphate, the proteid thereby precipitated was dissolved in water and the clear solution dialyzed. A small precipitate resulted, which when washed and dried, weighed one and four-tenths grams and had the following composition :

LUPIN GLOBULIN, 13.

Carbon	49.54
Hydrogen	6.91
Nitrogen	18.24
Sulphur } Oxygen }	25.31
	100.00
Ash	0.49

These fractional precipitations show a regular decrease in their content of carbon and an increase in both nitrogen and sulphur, that of the latter being especially marked. It is to be noted that the total weight of the foregoing fractions formed only 65.9 per cent. of the proteid taken, suggesting that the globulin was undergoing a change while in solution ; but as no especial care was exercised to obtain all the proteid from the solutions, this process was repeated, not only with a view to settling this point, but to obtain larger quantities of the extreme fractions for further examination.

One hundred grams of preparation 8 were dissolved in 800 cc. of five per cent. brine and the solution, after filtering perfectly clear, was mixed with 800 cc. of water, and cooled to 10°. The abundant precipitate which resulted was allowed to settle, and the solution decanted. The precipitate was marked *C*, the solution *D*. Precipitate *C* was next dissolved in 100 cc. of ten per cent. brine and 300 cc. of water at 20° added to the resulting

solution. After standing some time the clear solution was decanted from the large deposit of proteid and the latter washed and dried. Thus were obtained fifty grams of preparation 14, which analysis showed to have the following composition :

LUPIN GLOBULIN, 14.

Carbon	50.71
Hydrogen	7.00
Nitrogen	17.86
Sulphur	0.67
Oxygen	23.76
	100.00
Ash	0.39

The filtrate from 14 was mixed with 400 cc. of the first washings of 14 and cooled to 7°–8°. On standing, a part of the proteid deposited and the clear solution was then decanted. The precipitate, preparation 15, after washing and drying weighed 8.46 grams, and according to analysis contained :

LUPIN GLOBULIN, 15.

Carbon	50.14
Hydrogen	6.94
Nitrogen	17.83
Sulphur	0.86
Oxygen	24.23
	100.00
Ash	0.81

The solution from which 15 had separated was dialyzed, but only a very small quantity of globulin could be obtained, preparation 16, which weighed seven-tenths gram and without correction for ash contained 18.22 per cent. of nitrogen. Solution *D* decanted from precipitate *C*, as already described, was cooled to 3°, that is, 7° lower than before. This caused a further quantity of globulin to separate, which gave preparation 17, weighing 21.14 grams.

LUPIN GLOBULIN, 17.

Carbon	50.13
Hydrogen	6.88
Nitrogen	17.72
Sulphur	0.80
Oxygen	24.47
	100.00
Ash	0.59

The solution from which 17 separated was dialyzed free from chlorides and 13.7 grams of preparation 18 obtained, having the following composition :

LUPIN GLOBULIN, 18.

Carbon	50.04
Hydrogen	6.79
Nitrogen	18.43
Sulphur	1.48
Oxygen	23.26
	100.00
Ash	0.25

The filtrate from 18 contained but a very little proteid precipitable with ammonium sulphate. The total weight of these fractions was ninety-four grams; the six grams unaccounted for may be fairly attributed to mechanical loss and therefore a change of proteid to non-proteid substances is improbable.

These fractions, like those of the preceding series, show a decrease in carbon and an increase in nitrogen and sulphur as we pass from the least to the most soluble.

Another series of separations was next made by fractional solution.

One hundred grams of preparation 8 were dissolved in 800 cc. of five per cent. brine diluted with 800 cc. of water at 20° and the solution cooled to 5°. The clear solution was then decanted from the separated proteid and dialyzed till free from chlorine. The globulin thus separated after washing and drying weighed 24.03 grams and had the following composition :

LUPIN GLOBULIN, 19.

Carbon	50.11
Hydrogen	6.84
Nitrogen	18.46
Sulphur	1.28
Oxygen	23.31
	100.00
Ash	0.17

The proteid deposited by cooling, as just described, was dissolved in 700 cc. of five per cent. brine and cooled at 5°. The clear solution was decanted and dialyzed, yielding 18.60 grams of preparation 20, which contained :

LUPIN GLOBULIN, 20.

Carbon	50.27
Hydrogen	6.78
Nitrogen	18.43
Sulphur	1.15
Oxygen	23.37
	100.00
Ash	0.10

The substance precipitating on cooling, as just described, was dissolved in 600 cc. of five per cent. salt solution, mixed with an equal volume of water, and cooled to 2°. The clear solution was dialyzed and five and six-tenths grams of preparation 21 were obtained, which gave the following results on analysis:

LUPIN GLOBULIN, 21.

Carbon	50.03
Hydrogen	6.86
Nitrogen	18.47
Sulphur	1.49
Oxygen	23.15
	100.00
Ash	0.22

The precipitate that separated on cooling the solution from which 21 was obtained, was dissolved in 500 cc. of five per cent. brine, diluted with an equal volume of water, and cooled to 7°. The solution was decanted from the precipitate and dialyzed. Thus was obtained 4.31 grams of preparation 22, which contained:

LUPIN GLOBULIN, 22.

Carbon	50.44
Hydrogen	6.92
Nitrogen	18.23
Sulphur	1.14
Oxygen	23.27
	100.00
Ash	0.11

The substance deposited by cooling, as last described, was dissolved in 300 cc. of five per cent. salt solution, filtered clear and dialyzed till free from chlorides. The deposited globulin, after washing and drying, weighed 33.88 grams and contained:

LUPIN GLOBULIN, 23.

Carbon	51.13
Hydrogen	6.86
Nitrogen	18.03
Sulphur	0.49
Oxygen	23.49
	100.00
Ash	0.52

Like the preceding, this series of fractional separations shows a decrease in carbon and an increase in nitrogen and sulphur with increased solubility. The total weight of the fractions obtained was 86.42 grams, the loss being no greater than was to be expected.

Thirty-five grams of preparation 10 and the same quantity of preparation 14, representing the least soluble portions obtained in the two first series of fractions, were next dissolved in 600 cc. of five per cent. brine, diluted with an equal volume of water, and cooled to 6°. The solution was decanted from the precipitate which resulted, filtered clear, and dialyzed. The globulin thus precipitated after washing and drying weighed 12.43 grams and contained:

LUPIN GLOBULIN, 24.

Carbon	50.10
Hydrogen	6.94
Nitrogen	18.12
Sulphur	0.94
Oxygen	23.90
	100.00
Ash	0.23

The precipitate produced by cooling the solution, as just described, was dissolved in 500 cc. of salt solution, diluted with 500 cc. of water and cooled to 6°. The deposited proteid, after the usual treatment, weighed 7.80 grams and had the following composition:

LUPIN GLOBULIN, 25.

Carbon	50.32
Hydrogen	6.90
Nitrogen	18.06
Sulphur	0.81
Oxygen	23.91
	100.00
Ash	0.27

The substance separated by cooling, as just described, was dissolved in one liter of two and one-half per cent. sodium chloride solution and cooled to 7°. The clear liquid, decanted from the thus precipitated proteid, was dialyzed and yielded 5.51 grams of globulin, giving, on analysis, the following results:

LUPIN GLOBULIN, 26.

Carbon	50.80
Hydrogen	6.91
Nitrogen	18.01
Sulphur	0.64
Oxygen	23.64
	100.00
Ash	0.23

The proteid separated by cooling the solution from which 26 had been obtained was dissolved in half a liter of five per cent. salt solution, diluted with an equal volume of water, and cooled to 14°. The clear solution was decanted and dialyzed and gave four and nine-tenths grams of preparation 27, which analysis showed to have the composition here given:

LUPIN GLOBULIN, 27.

Carbon	50.90
Hydrogen	6.85
Nitrogen	17.88
Sulphur	0.55
Oxygen	23.82
	100.00
Ash	0.27

The substance deposited at 14°, as noted above, was dissolved in half a liter of five per cent. brine, diluted to one liter, and allowed to settle at the temperature of the room (about 22°). The liquid was then decanted, dialyzed, and further treated in the usual manner. There was thus obtained 5.45 grams of preparation 28, which contained:

LUPIN GLOBULIN, 28.

Carbon	50.93
Hydrogen	6.94
Nitrogen	17.83
Sulphur	0.48
Oxygen	23.82
	100.00
Ash	0.23

The substance separated by diluting the solution from which 28 resulted, was washed with water and alcohol, dried, and found to weigh 20.6 grams. Its composition was :

LUPIN GLOBULIN, 29.

Carbon	50.80
Hydrogen	6.83
Nitrogen	17.88
Sulphur	0.46
Oxygen	24.03
	100.00
Ash	0.70

This, like the other series of fractional separations, shows an increase in carbon and decrease in sulphur and nitrogen content accompanying a decrease in solubility. If the final fractions, which have been most thoroughly purified, are arranged as in the table below, it will be seen that a nearly constant composition has been reached so that the average of these analyses may be taken as closely representing the composition of the least soluble and most abundant globulin of the yellow lupin.

YELLOW LUPIN GLOBULIN, CONGLUTIN.

	23.	26.	27.	28.	29.	Average.
Carbon	51.13	50.80	50.90	50.93	50.80	50.91
Hydrogen	6.86	6.91	6.85	6.94	6.83	6.88
Nitrogen	18.03	18.01	17.88	17.83	17.88	17.93
Sulphur	0.49	0.64	0.55	0.48	0.46	0.52
Oxygen	23.49	23.64	23.82	23.82	24.03	23.76
	100.00	100.00	100.00	100.00	100.00	100.00

This is the globulin discovered by Ritthausen, and described by him under the name conglutin.

A careful examination of preparation 29 showed this globulin to have the following reactions :

In very dilute acids and alkalies it is completely soluble, yielding perfectly clear solutions of a light yellow color.

Dissolved in very dilute acetic acid, the proteid is precipitated by neutralizing the solution with sodium carbonate. On adding sodium chloride brine to the solution containing the precipitate in suspension, the latter is completely dissolved.

In ten per cent. sodium chloride brine it dissolves readily, giving a very slight turbid solution of a pale yellow color, which

turns litmus paper red, lacmoid paper blue, and has no effect on tropæoline when evaporated to dryness with it.

A solution containing ten per cent. of the globulin dissolved in ten per cent. sodium chloride brine behaved as follows :

Dilution with twice its volume of water produced a considerable precipitate.

Addition of one drop of acetic acid, sp. gr. 1.035, or one drop of hydrochloric acid (one part concentrated acid and three parts of water) to five cc. of this solution gave a heavy precipitate.

Addition of mercuric chloride[1] dissolved in ten per cent. brine gave no precipitate.

Tannin as well as picric acid gave a heavy precipitate.

Diluted with an equal volume of ten per cent. brine the solution, containing five per cent. of globulin, reacted as follows :

No precipitate was produced by saturation with sodium chloride, magnesium sulphate or sodium sulphate at 20°, but at 34° saturation with the last named salt precipitated all but a minute trace of the globulin.

When the solution in ten per cent. sodium chloride brine, containing five per cent. of proteid, was heated gradually in a water-bath, no change appeared even after heating some time at 100°. After more prolonged heating a thick transparent skin formed on the surface. On cooling and standing several hours, the solution set to a solid jelly and became somewhat turbid.

Addition of nitric acid to the solution in brine gave a precipitate not soluble in an excess of this acid. On heating, the usual xanthoproteic reaction occurred, which was preceded by the development of a slight pink color quickly changing to yellow, doubtless due to a trace of coloring matter still adhering to the proteid. When the globulin in the dry state was treated with very dilute nitric acid a clear solution resulted which gave a heavy precipitate on adding an excess of acid, that behaved on heating as just described. The usual proteid reactions were obtained with Millon's, Adamkiewics', and the biuret tests.

In order to determine the composition of the more soluble fractions, the greater parts of 18, 19, and 20 were united, giving

[1] Palladin (Ztschr. für Biol., **31**, 195) states that ten per cent. sodium chloride solutions are not precipitated by mercuric chloride, but that diluted solutions give precipitates soluble in salt solution. The precipitate which he thus obtained was of course caused by the water added with the mercuric chloride. If he had dissolved the mercury salt in brine, no precipitate would have resulted.

forty grams of substance which was dissolved in 400 cc. of five per cent. brine, the solution was filtered perfectly clear, the filter washed with 100 cc. of the same salt solution, and the filtrate and washings were mixed with an equal volume of water. After cooling to 8°, and standing some time, a part of the globulin separated. From this the solution was decanted. The proteid was deposited as a viscid transparent fluid, which became opaque on treating with distilled water and finally solid. It was dehydrated with absolute alcohol and dried over sulphuric acid, giving 19.2 grams of preparation 30, having the following composition :

LUPIN GLOBULIN, 30.

Carbon	49.64
Hydrogen	6.87
Nitrogen	18.21
Sulphur	1.20
Oxygen	24.08
	100.00
Ash	0.32

The solution decanted from preparation 30 was mixed with 500 cc. of water and cooled to 7°. The resulting precipitate, when treated in the same way as 30 had been, gave 9.25 grams of preparation 31, containing :

LUPIN GLOBULIN, 31.

Carbon	49.62
Hydrogen	6.72
Nitrogen	18.22
Sulphur	1.36
Oxygen	24.08
	100.00
Ash	0.43

The solution from which 31 had separated was dialyzed free from chlorides, and yielded seven and four-tenths grams of preparation 32 :

LUPIN GLOBULIN, 32.

Carbon	49.59
Hydrogen	6.75
Nitrogen	18.43
Sulphur	1.62
Oxygen	23.61
	100.00
Ash	0.17

These three fractions have very nearly the same composition, which is in close agreement with that of the other most soluble fractions already described, as may be seen from the following table :

LUPIN GLOBULIN.

	13.	18.	9.	30.	31.	32.	Average.
Carbon	49.54	49.63	49.47	49.64	49.62	49.59	49.58
Hydrogen...	6.91	6.78	6.77	6.87	6.72	6.73	6.80
Nitrogen....	18.24	18.43	18.08	18.21	18.22	18.43	18.27
Sulphur .. }	25.31	1.48	1.49	1.20	1.36	1.56	1.42
Oxygen... }		23.68	24.20	24.08	24.08	23.67	23.93
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

A series of tests of preparation 30, conducted at the same time and under identical conditions with those described for preparation 29, revealed the following differences. In ten per cent. sodium chloride brine, 30 dissolved to a perfectly clear solution more colored than that yielded by 29.

Preparation 30, dissolved in brine to a solution containing ten per cent. both of salt and proteid, was not even rendered turbid by dilution with two volumes of water, but with three volumes a slight precipitate was given. A similar solution of 29, with two volumes of water, gave a considerable precipitate, and with three volumes a very heavy precipitate.

Five cc. of the ten per cent. solution of 30 required eight drops of acetic acid, sp. gr. 1.035, to produce a slight precipitate, while one drop under the same conditions gave a heavy precipitate in a similar solution of 29.

With one drop of hydrochloric acid (one part concentrated hydrochloric acid and three parts water) only a turbidity was produced in the case of 30, while under like conditions 29 yielded a heavy precipitate.

Saturation with sodium sulphate of the solution containing five per cent. of 30 gave only a partial precipitation, 29 being wholly precipitated under like conditions.

When this five per cent. solution in ten per cent. brine was heated in a water-bath a turbidity formed at 94°, increasing as the temperature was raised, becoming dense at 99°, and after long heating a gelatinous flocculent precipitate separated which was unchanged on cooling and standing ; when heated with

nitric acid the pink color which preceded the yellow of the xanthoproteic reaction was more pronounced than that yielded by 29.

In all other respects no difference could be detected between the reactions of 30 and 29. The most noticeable property in which both differ from nearly all other vegetable globulins is that neither yields insoluble products (the so-called "albuminates") when separated from solution. The only evidence of such a tendency noticed in the very large number of preparations made, was in some cases shown by the presence of a slight turbidity when precipitates were redissolved.

Both give solutions which react strongly acid with litmus. Titrated with standard ammonia, two grams of each of these globulins required the addition of ten milligrams of ammonia to cause an alkaline reaction with litmus.

Although the analyses of the final fractions of the more soluble globulin agree closely and their properties and reactions are quite alike, it seems to us doubtful if they represent a definite chemical species. The close physical and chemical resemblance between the least soluble substance and the most soluble, suggests that they are closely related. The distinct difference in some properties observed between the extreme products of fractional precipitation make it probable that a combination of some sort has taken place between the conglutin and other constituents of the seed.

As the lupin seed is unusually rich in soluble constituents, it would not be surprising if the proteid on precipitation carried down with it more or less of these substances which could be with difficulty separated afterwards. From the globulin of the blue lupin by fractional precipitation readily soluble products were obtained which however were wholly different in composition and quite distinct in reaction from the more soluble fractions of the yellow lupin globulin. The soluble fractions are possibly conglutin combined either chemically or mechanically with other constituents of the seed, which in the two varieties of lupin are apparently present in different proportions.

III. *Proteid Insoluble in Water and Saline Solution but Soluble in Dilute Alkalies.*

As previously described on page 457 after exhausting one kilogram of yellow lupin meal with ten per cent. sodium chloride

solution and washing the residue with water, by continued extraction with two-tenths per cent. potash water a solution was obtained which after filtering clear and adding acetic acid to distinct acid reaction yielded a precipitate which after washing with water and alcohol and drying over sulphuric acid weighed 60.53 grams. This was redissolved in two-tenths per cent. potash water, the solution again filtered clear and carbon dioxide passed through it. The proteid partly separated, but could not be filtered, so a little ammonium chloride was added to convert the potassium carbonate into chloride, but even after passing carbon dioxide through the solution for some time and allowing it to stand over night in an ice box, only a partial separation resulted. Acetic acid was then added in slight excess and the precipitate filtered off and washed thoroughly with water and with alcohol. Dried at 110° this preparation, 33, gave the following results on analysis :

LUPIN PROTEID, 33.

Carbon	51.40
Hydrogen	6.79
Nitrogen	16.43
Sulphur	1.03
Oxygen	24.35
	100.00
Ash	1.57

BLUE LUPIN.

One kilogram of fine ground meal of the seeds of the blue lupin was extracted with a large quantity of distilled water applied in successive portions and the residual meal thrown on fine bolting cloth after each application. The extract thus obtained was allowed to stand over night. The partly clarified liquid was siphoned off, saturated with ammonium sulphate, the precipitate dissolved in brine, and the solution filtered clear and dialyzed for forty-eight hours. A precipitate resulted which was filtered off, washed with water and with alcohol, and dried over sulphuric acid, giving preparation 34, weighing 8.46 grams. The filtrate was dialyzed for ten days longer in a stream of water, but no more proteid separated. The solution was then concentrated by dialysis in alcohol and absolute alcohol added until all the proteid contained in it was precipitated. The sub-

stance thus obtained weighed, when dry, only 1.42 grams and was not examined further than to find that it was nearly all insoluble in water, having been coagulated by the prolonged treatment with alcohol. This insolubility of the greater part of this substance shows that very little proteose is present.

Like the yellow lupin, this seed contains but little proteid matter soluble in water, and that is mostly globulin dissolved by aid of the salts extracted from the seed.

The meal residue which had been exhausted with water was treated with ten per cent. sodium chloride solution and the extract, after filtering perfectly clear, was dialyzed for forty hours. The globulin, which separated in a coherent mass on the bottom of the dialyzer, was washed thoroughly by decantation with water and with alcohol and dried over sulphuric acid. This gave 115.0 grams of preparation 35.

Another preparation of this globulin was made by treating one kilogram of the meal directly with ten per cent. brine, filtering the extract perfectly clear, and dialyzing for forty hours. The globulin thus precipitated, after washing and drying, formed preparation 36 and weighed 112 grams. These three preparations were dried at 110° and analyzed with the following results:

BLUE LUPIN GLOBULIN, CONGLUTIN.

	34.	35.	36.	Yellow lupin.
Carbon	50.58	50.82	50.85	50.91
Hydrogen	6.58	6.87	6.78	6.88
Nitrogen	17.82	18.04	18.04	17.93
Sulphur	0.72	0.40	0.50	0.52
Oxygen	24.30	23.87	23.83	23.76
	100.00	100.00	100.00	100.00
Ash	0.64	1.22	1.11	

The agreement between 35 and 36 and the purified conglutin of the yellow lupin is very close indeed, but in order to be sure that this was not accidental these two preparations were subjected to the following treatment:

One hundred grams of 35 were dissolved in one-half liter of five per cent. sodium chloride brine and one-half liter of water added to the solution. A large rapidly settling precipitate resulted, which formed a semi-fluid mass on the bottom of the beaker, from which the very nearly clear solution *A* was poured

after a short time. The precipitate *B* was dissolved in 280 cc. of six and a half per cent. brine and the resulting solution, which measured 380 cc., was diluted with an equal volume of water and cooled to 5°. The solution was then decanted from the precipitate, which was washed and dried as usual and found to weigh 39.2 grams, preparation 37. The solution decanted from 37 was dialyzed until free from salt, whereby a precipitate resulted which, when washed and dried, weighed four and two-tenths grams and formed preparation 38. The solution *A*, decanted from precipitate *B*, as above described, was cooled to 5° and the solution *C* decanted from the precipitate *D*. This precipitate was again dissolved in 300 cc. of five per cent. brine, 300 cc. of water added, and the whole cooled to 5°. The precipitate thus produced, when dried, weighed 19.5 grams, preparation 39. The solution from which 39 had separated was dialyzed and yielded preparation 40, weighing five and four-tenths grams.

The solution *C* decanted from precipitate *D* was dialyzed free from chlorides and thereby thirteen grams of preparation 41 were separated. These preparations were dried at 110° and analyzed with the following results. In the table they are arranged according to their solubility in dilute salt solutions.

	37.	39.	38.	40.	41.
Carbon	51.25	51.04	50.82	50.98	50.79
Hydrogen	6.96	6.75	6.66	6.83	6.79
Nitrogen	18.11	18.15	17.69	17.66	17.64
Sulphur	0.32	0.24	0.49	0.38	0.39
Oxygen	23.36	23.82	24.34	24.15	24.39
	100.00	100.00	100.00	100.00	100.00
Ash	0.95	0.71	0.61	0.52	0.75
Amount	39.2	19.5	4.2	5.4	13.0 grams

From these figures it would seem that preparation 35 contained some impurities, which accumulated in the three most soluble fractions in which the nitrogen is a little lower and the sulphur slightly higher than in the less soluble fractions. Preparation 36 was next fractionally precipitated in the following manner:

One hundred grams were dissolved in 700 cc. of five per cent. sodium chloride brine, filtered perfectly clear, and the solution diluted with an equal volume of distilled water. The proteid

separated as a viscid liquid from which the solution *E* was decanted. The precipitate *F* was dissolved in 100 cc. of five per cent. brine and the resulting solution, which measured 175 cc., was cooled in a freezing mixture to —4°, and then allowed to stand until it had warmed to 20°. The precipitate thus produced formed a perfectly transparent syrupy liquid which measured 61 cc. The solution from which this had separated was decanted and the fluid precipitate was washed by stirring up with water, whereby it was rendered opaque and pasty. On washing with fresh quantities of water the substance became denser and granular. It was finally washed with alcohol and dried over sulphuric acid, giving twenty-five grams of preparation 42.

The washings of 42 were cooled to 0° and four-tenths gram of preparation 43 obtained. The solution from which this separated contained very little proteid, which was not saved. The solution *E*, decanted from precipitate *F*, was cooled to 8°, and the solution *G*, was decanted from the proteid *H*, thus separated.

This precipitate was treated with seventy-seven cc. of five per cent. sodium chloride brine giving a solution measuring 126 cc., which was cooled to —10°, and then allowed to stand until warmed to 20°, when forty-nine cc. of a clear viscid liquid separated. This was washed with water in the same manner as 42 had been, and then with alcohol, dried over sulphuric acid, and 18.5 grams of preparation 44 obtained. From the washings of 44 by cooling to 0°, 2.62 grams of preparation 45 were separated. The solution *G*, decanted from precipitate *H*, was cooled in a freezing mixture until partly frozen, when it was allowed to melt and deposit the separated proteid. The latter, *J*, after decanting the solution *I*, was dissolved in fifty cc. of five per cent. sodium chloride brine and the seventy-five cc. of solution which resulted was cooled to —2°, but only a turbidity resulted. The solution was therefore diluted with an equal volume of water, again cooled to —2°, and slowly warmed to 20°. The proteid separated as a viscid liquid measuring twenty-four cc., and when washed with water and alcohol gave ten grams of preparation 46. From the washings of 46, by cooling to 0°, there separated 0.56 gram of preparation 47. The solution *I*,

decanted from precipitate *J*, was diluted with an equal volume of water, and cooled to 0°. The substance which separated was washed with water and alcohol and when dried weighed 9.15 grams, and formed preparation 48. The solution from which 48 had separated contained too little proteid to save.

Dried at 110° these preparations gave the following results on analysis, which are arranged in the table in the order of their solubility.

	42.	44.	43.	45.	46.	47.	48.	
Carbon....	51.09	51.14	....	50.86	50.94	....	50.65	
Hydrogen.	6.83	6.89	....	6.89	6.89	....	6.84	
Nitrogen..	18.08	18.10	17.82+	17.95	17.79	17.77	17.56	
Sulphur ..	0.38	0.33	....	0.46	0.27	....	0.44	
Oxygen...	23.62	23.54	....	23.84	24.11	....	24.51	
	100.00	100.00		100.00	100.00		100.00	
Ash	0.59	0.47	?	0.69	0.51	0.54	0.86	
Amount ..	25.00	18.50	0.40	2.62	10.00	0.56	9.15	gms.

If the analyses of preparations 37, 39, 42, and 44, which constitute the greater part of the proteid substance of 35 and 36, are compared it will be seen that they are in very close agreement, and it is fair to presume that they represent very nearly the true composition of this, the principal proteid of the blue lupin. If these analyses are also compared with those of conglutin from the yellow lupin, it will be evident that the two varieties of lupin contain one and the same globulin, especially since a rigid comparison of the reactions of purified preparations from the two seeds failed to reveal the slightest difference. The following table will facilitate a comparison of the above-mentioned figures:

CONGLUTIN.

	Blue lupin.					Yellow lupin.
	37.	39.	42.	44.	Average.	Average.
Carbon	51.25	51.04	51.09	51.14	51.13	50.91
Hydrogen....	6.96	6.75	6.83	6.89	6.86	6.88
Nitrogen.....	18.11	18.15	18.08	18.10	18.11	17.93
Sulphur	0.32	0.24	0.38	0.33	0.32	0.52
Oxygen.....	23.36	23.82	23.62	23.54	23.58	23.76
	100.00	100.00	100.00	100.00	100.00	100.00

If now we compare the analyses of the more soluble fractions, as shown in the following table, they will be seen to be quite

similar to each other but decidedly different from the more soluble globulin of the yellow lupin:

	38.	40.	41.	46.	47.	48.	Average.	Soluble globulin yellow lupin.
Carbon ...	50.82	50.98	50.79	50.94	....	50.65	50.84	49.58
Hydrogen.	6.66	6.83	6.79	6.89	....	6.84	6.80	6.80
Nitrogen .	17.69	17.66	17.64	17.79	17.77	17.56	17.69	18.27
Sulphur ..	0.49	0.38	0.39	0.27	....	0.44	0.39	1.42
Oxygen ...	24.34	24.15	24.39	24.11	....	24.51	24.28	23.93
	100.00	100.00	100.00	100.00		100.00	100.00	100.00

A comparison of the reactions of 41 with those of 37 showed that much less difference existed between the extreme fractions from the blue lupin than between those from the yellow. A ten per cent. solution of 37 in ten per cent. brine gave a considerable precipitate when diluted with twice its volume of water, while three times its volume were required to produce a slight precipitate in similar solutions of 41. Solutions of both were precipitated with equal quantities of acid, 41 not needing the large excess of acid to cause precipitation which the soluble product from the yellow lupin required.

A solution of five per cent. of 37, in ten per cent. brine, even after prolonged heating at 99°–100°, appeared wholly unaffected until the solution was subsequently cooled, when it solidified. A similar solution of 41 began to yield a flocculent coagulum at 75°, which at 80° was voluminous. After heating in a boiling water-bath for some time nearly all the proteid was coagulated. As to the relations of these two substances, what was said on page 474, in our opinion, applies equally in this case.

CONCLUSION.

Both yellow and blue lupin seeds contain very little proteid matter soluble in water. The total quantity of proteid soluble in pure water obtained from the yellow lupin amounted to only 0.37 per cent. Of this a part consists of proteose. Whether the remainder is albumin, or a globulin soluble in extremely dilute salt solutions, which therefore could not be completely separated by dialysis, was not determined. Peptone is not contained in the freshly ground seed but is formed in small quantity after prolonged contact with water.

The greater part of the proteid matter contained in these seeds is soluble in saline solutions, the yellow lupin yielding 26.2 per cent. This is the body known as conglutin, but as heretofore described and as usually obtained it is contaminated with other substances present in the seed. Preparations from the blue lupin are usually much purer than those from the yellow, for the latter contain a considerable quantity of some sulphur-containing substance from which conglutin can be separated by fractional precipitation out of dilute salt solutions. This explains why Ritthausen's conglutin from the yellow lupin contained twice as much sulphur as that from the blue lupin.

When purified no difference in properties and reactions can be detected between preparations from the two seeds.

The composition of conglutin, as obtained by us, is shown by the following figures:

CONGLUTIN.

	Yellow lupin.	Blue lupin.
Carbon	50.91	51.13
Hydrogen	6.88	6.86
Nitrogen	17.93	18.11
Sulphur	0.52	0.32
Oxygen	23.76	23.58
	100.00	100.00

Conglutin is readily soluble in sodium chloride solutions containing upwards of five per cent. of the salt. By sufficient dilution it is precipitated, a syrupy liquid separating which is rendered opaque and solid by treatment with water. Dissolved in salt solution, it is apparently unaffected by prolonged heating in a boiling water-bath, but solutions thus heated on standing and cooling form a solid opalescent jelly which becomes clear and fluid on again heating. Unlike other globulins conglutin does not yield insoluble (coagulated) products by washing with alcohol or drying.

After exhausting lupin meal with salt solution, a small quantity of proteid can be extracted by two-tenths per cent. potash water, from which it is precipitated by adding acetic acid in slight excess but not by making the solution neutral to litmus. Only one preparation of this substance was made, which gave the following results on analysis:

Carbon	51.40
Hydrogen	6.79
Nitrogen	16.43
Sulphur	1.03
Oxygen	24.35
	100.00

Owing to the insolubility of this substance in any but alkaline fluids and the difficulty of making preparations of known purity, nothing further was learned respecting it.

EFFECT OF MINUTE QUANTITIES OF ACID ON THE SOLUBILITY OF GLOBULIN IN SALT SOLUTIONS.[1]

By Thomas B. Osborne and George F. Campbell.

Received April 12, 1897.

IN a paper on crystallized vegetable proteids by one of us[2] it is shown that the principal globulin of the seed of the castor bean is partly insoluble in a saturated solution of sodium chloride, and partly soluble therein, and that these two parts are alike in composition and but slightly different in reactions. Having found a proteid of similar composition and properties in the sunflower seed, we have again turned our attention to the globulin of the castor bean, with the hope that we might discover the cause of this partial precipitation by saturating its solutions with salt.

A considerable quantity of this globulin was prepared by extracting castor pomace with three per cent. brine at 60° and allowing the filtered extract to cool to the temperature of the room. The proteid thus separated was washed with water and alcohol, and dried over sulphuric acid. It formed a slightly colored dense powder consisting of a mixture of spheroids and octahedral crystals.

Seventy-five grams of this preparation were treated with 750 cc. of ten per cent. salt solution, and after agitating for some time, filtered from a large insoluble residue. This latter was washed thoroughly with ten per cent. brine and the filtrate and washings were united. In this way the substance was separated into two parts, one soluble and one insoluble, in cold salt solution. This solution was then saturated with sodium chloride

[1] From the Report of the Connecticut Agricultural Experiment Station for 1896.

[2] Osborne: *Am. Chem. J.*, 14, 671.

and the large precipitate produced was filtered off, dissolved in 10 per cent. brine and this process twice repeated. The saturated sodium chloride solutions filtered from these precipitations were united and dialyzed free from salt; the proteid thus precipitated was washed with water and alcohol, and dried over sulphuric acid. Thus 12.39 grams of preparation A were obtained, representing the fraction of this globulin soluble in cold ten per cent. brine and *not* precipitated by saturation with sodium chloride. The proteid which had been several times precipitated from solution by saturation with salt, as just described, was dissolved in ten per cent. brine, and the solution filtered perfectly clear and dialyzed. By the usual treatment 18.52 grams of preparation B were obtained, representing the part of this globulin soluble in cold ten per cent. brine, but insoluble in saturated brine.

The part of the globulin which failed to dissolve in cold ten per cent. sodium chloride solution was next treated with salt solution of this strength, heated to 60° and allowed to cool to 14°. The greater part of the proteid was dissolved by this treatment, and after decanting the solution the undissolved residue was treated three successive times in the same way. The solutions obtained by this process were filtered clear from a slight quantity of suspended matters and saturated with sodium chloride, which precipitated all but an insignificant quantity of the dissolved proteid.

This precipitate was dissolved in ten per cent. brine, filtered clear and dialyzed. The precipitated globulin, after filtering off, washing, and drying, weighed 12.37 grams, and formed preparation C.

The part of the original globulin which failed to remain in solution after the above treatment with hot salt solution was dissolved in brine at 60°, filtered clear, and allowed to cool over night. Very nearly all the proteid precipitated on cooling, and was washed and dried, giving preparation D, weighing 1.66 grams.

These four preparations were analyzed with very great care with the following results :

	A.	B.	C.	D.
Carbon	50.99	51.10	51.12	51.25
Hydrogen	6.92	6.87	6.95	6.97
Nitrogen	18.95	18.67	18.83	18.74
Sulphur } Oxygen }	23.14	23.36	23.10	23.04
	100.00	100.00	100.00	100.00
Ash	0.19	0.14	0.14	0.41

The difference between these results barely exceed the usual errors of analysis, although several determinations of each element in the different fractions indicate that these differences are not due to analytical errors. It would not be safe, however, to take such slight variations into account, especially when we consider the great difficulty in making perfectly pure preparations of proteids as well as exact combustions. We must, therefore, conclude that no difference in composition is proved to exist between these four preparations which present such marked differences in solubility. A comparative examination of these substances was made with the following results:

In ten per cent. brine at 20°, A dissolved completely, B with the exception of a small residue, C partly, much being insoluble, while D did not dissolve at all. B and C dissolved nearly completely when warmed to 45°.

Five grams of each of A, B, and C were dissolved in 50 cc. of ten per cent. sodium chloride solution by heating to 50°. On cooling to 20°, B and C deposited a very slight amount of proteid, but on cooling to 12°, A gave a clear solution, while B deposited a not inconsiderable quantity of substance and C decidedly more. To five cc. of each of these solutions at 20° were added five cc. of water. A remained clear, B gave a slight precipitate, and from C practically all the dissolved globulin was thrown down, since further dilution of the solution filtered from this precipitate gave only a turbidity. With five cc. more water added to A a turbidity resulted, while the same amount added to B gave a heavy precipitate. The dilution of A and B was continued until the strength of the salt solution was 1.66 per cent., when B yielded no more by further dilution, and A still contained dissolved globulin.

Five per cent. solutions of each of these proteids, in ten per cent. brine, when heated became turbid. A and B at 88°, C at

87°, and flocculent coagula formed in A and C at 90° and in B at 91°, thus showing no difference in relation to heat.

Saturation with sodium chloride of the ten per cent. solution of this globulin gave a small precipitate in A, but completely precipitated B and C.

This partial precipitation of A shows that the substance precipitated by saturating with salt is a derivative of the body originally soluble in saturated brine.

In order to find the effect of minute quantities of acid added to these several fractions, two grams of each were treated with 20 cc. of 0.05 per cent. acetic acid, or five milligrams of acid for each gram of proteid, which caused no noticeable solution. Two grams of salt were added to each whereby A was largely, B partly, and C but slightly dissolved. Heated to 50°, A gave a clear solution, B a nearly clear solution, while C dissolved only partly and precipitated on cooling to 20°.

The acid added to these solutions could not be detected by very delicate litmus paper, the reaction being perfectly neutral.

A solution of A was prepared in exactly the same manner, omitting the acetic acid, and the two solutions compared.

Diluted with an equal volume of water, no precipitate formed in either solution, but with two volumes an abundant precipitate fell in that containing the acid, while only a very slight precipitate formed in the other. Saturated with sodium chloride, the solution with acid gave a large precipitate, that without acid only a small one.

It is thus clear that a quantity of acid too small to be detected with litmus or by analysis causes changes in the fractions soluble in saturated salt solution, whereby products result having the same general properties as those exhibited by the fractions B and C.

In order to obtain more evidence on this point, these experiments were repeated and extended, using crystallized edestin from hemp seed.

Five grams of edestin were suspended in 50 cc. of 0.05 per cent. acetic acid, five grams of salt were added, and the solution was warmed to 50° to dissolve the insoluble "albuminate" present.

Another solution was then prepared in exactly the same man-

ner without using acetic acid. Both solutions reacted perfectly neutral to litmus paper.

Equal volumes at the same temperature, and in test-tubes of the same size were immersed in the same bath of cold water. The solution containing acid precipitated first and in far greater amount than the other.

Five cc. of each were diluted with an equal volume of water at 20°. The solution with acid gave a much greater precipitate than the other. After allowing these to stand and cool to 10°, the precipitates were filtered off and one drop of strong acetic acid was added to each filtrate. The solution which had been made with acid gave only a turbidity, while a considerable precipitate formed in the other, showing that dilution precipitates the solution to which the acid had been added far more readily than the solution without the acid.

Equal volumes of these solutions were saturated with salt, the precipitates filtered off, and one drop of acetic acid was added to each filtrate. That from the solution made with acid gave only a turbidity, while the other gave a very heavy precipitate.

Here again we see that the addition of a quantity of acid, too small to detect after the solution has been made, brings about changes similar to those naturally occurring in the seeds and extracts of the castor bean and sunflower and to those following the addition of acid to that part of the globulin of the castor bean which is soluble in saturated salt solutions.

Whether such changes occur only through the influence of acids is a question not settled, and regarding which some doubt is raised by the fact that preparations of crystallized edestin which were originally soluble in ten per cent. sodium chloride solution with the exception of a small quantity of "albuminate" and yielded solutions which gave only traces of precipitates on saturating with sodium chloride, were found, after keeping dry and in cork-stoppered bottles two and four years, to have become largely insoluble in cold salt solution and to yield solutions which were nearly completely precipitated by saturating with salt. The insoluble portion dissolved nearly completely in ten per cent. brine at 60° to a solution precipitated somewhat by cooling to 20°, and abundantly at lower temperatures, copiously precipitated by dilution with an equal volume of water, and

almost completely precipitated by saturating with sodium chloride. It is not at all impossible that this change, too, may have been caused by acid, for these preparations stood for several years in the laboratory, the air of which at times contained some acid vapors. We thus see the same change taking place in the dry proteid on long keeping as those definitely caused by minute quantities of acid.

That this change to a condition in which the globulin is precipitated by salt is an intermediate step towards the formation of the insoluble form, the so-called "albuminate" of Weyl is evident from what has already been stated, especially the fact that by treatment with warm salt solution this insoluble matter can be changed into the form soluble in cold salt solution and precipitable by saturation with salt.

In this connection it is interesting to note that the only animal globulin obtained from an acid tissue is myosin, and that this myosin not only is readily precipitated by saturating with salt, but quickly and spontaneously changes to the insoluble form known as syntonin. In the dead muscle the amount of acid greatly exceeds that used in our experiments, for its presence is plainly shown by the strong acid reaction of the muscle serum. In alkaline muscle plasma myosin is not found, but myosinogen, paramyosinogen, and myoglobulin. The last three are described as precipitated by saturation with sodium chloride, but it may be that when tested in this respect the formation of acid had already begun and had reached a point where it caused precipitation with salt, but could not be detected by the usual tests.

THE PROTEIDS OF THE SUNFLOWER SEED.[1]

By Thomas B. Osborne and George F. Campbell.

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THE only published observations on the proteid of the sunflower seed which we have found were made by Ritthausen[2] and by Vines.[3] By extracting with very dilute alkali Ritthausen obtained from finely ground oil-free meal 44.71 per cent. of proteid, having the composition given under 1. By treating with sodium chloride brine, diluting the extract with

[1] From the Report of the Connecticut Agricultural Experiment Station for 1896.

[2] Pflüger's Archiv., **21**, 89, 1880.

[3] *J. Physiol.*, **3**, 93.

five volumes of water and passing carbon dioxide through the solution he got 25.3 per cent. with the composition given under 2, and by exhausting a preparation obtained in the same manner as 1 with brine, and proceeding as with 2, he got a preparation whose analysis is given under 3.

Sunflower Proteid.	Ritthausen.		
	1.	2.	3.
Carbon	51.88	51.51	51.18
Hydrogen	6.66	6.76	6.82
Nitrogen	17.99	18.21	18.06
Sulphur	0.71	0.61	
Oxygen	22.76	22.91	23.94
	100.00	100.00	100.00

Vines states that if a section of sunflower seed be treated with ether to remove oil it will be found that the aleurone grains, though readily soluble in ten per cent. sodium chloride solution, will not dissolve in saturated solution; if, however, they be treated with alcohol instead of ether, the globulin of which these grains consist behaves like vitellin, that is, it dissolves in a saturated solution of sodium chloride.

Vines further states that "it is of interest to note the fact that most of the substances which I found in the grain recur in the crystalloids, more especially vitellin and its derivatives; thus the peculiar globulin which forms the crystalloids of Ricinus appears to be in the grains of Helianthus."

Ritthausen's results indicate that by far the greater part of the proteid matter of the sunflower seed has a uniform composition, and that a large part of this proteid is insoluble in salt solution, but soluble in dilute alkali. The composition which he found for this proteid resembles that of the globulin edestin, which we have found in many seeds, the only difference being a slightly lower content of nitrogen. On this account it seemed to us desirable to examine this proteid and determine its relation to edestin. This was the more important, because Vines' statement of its behavior when treated with ether and with alcohol showed it to possess the same peculiar relations to salt solutions observed by one of us[1] in studying the globulin of the castor bean, *Ricinus communis*. As the deportment of globulins to saturated sodium chloride solutions has been made the basis of

[1] Osborne: Report Conn. Expt. Station, 1892, p. 138, and *Am. Chem. J.*, **14**, 671.

a division of these bodies into two main classes, it is important for us to know whether this is founded on fundamental differences in the proteids, or is simply due to the unlike conditions under which the proteids are found.

Sunflower seeds were crushed and a large part of the woody shells removed. The meal was then ground under benzine and after freeing from oil, air-dried. This meal when treated with ten per cent. sodium chloride brine yielded an extract of a strong blackish green color, from which a considerable quantity of proteid could be separated by dilution, by dialysis, or by saturation with sodium chloride.

When heated, this extract becomes turbid at 48° and flocks separated at 62°. The solution heated to 75° and filtered from this slight coagulum yielded a large precipitate when saturated with salt, thus showing that most of the substance thus precipitated is not, as *myosin* is said to be, coagulated below 75°.

The unheated extract saturated with sodium chloride gives a precipitate which when dissolved in ten per cent. brine coagulates at the same temperature as the original extract, but the amount of this coagulum is but a small fraction of the substance precipitated by saturation with salt.

The following preparations were made, but as subsequently pointed out, were found to be more or less impure, so that these results have value only as affording evidence of the uniform composition of the globulin extracted by salt solution from the sunflower seed.

	1	2	3	4	5
Carbon	51.57	51.77	51.65	51.69	51.85
Hydrogen	6.81	6.83	6.72	6.80	6.84
Nitrogen	18.16	18.20	18.17	18 24	18.00
Sulphur }	23.46	23.20	23.46	0.78	23.21
Oxygen }				21.49	
	100.00	100.00	100.00	100.00	100.00

	6	7	8	9
Nitrogen	18.20	18.23	18.09	18.07

Of these, 1 is the total globulin extracted by brine from one portion of oil-free meal; 2, 3, 4, and 5 are fractional precipitates from another similar extraction; 6, substance precipitated by saturating the salt extract with sodium chloride; 7 and 8, substance soluble in saturated sodium chloride solution; and 9, that

precipitated by cooling an extract made with a one and a half per cent. salt solution heated to 60°.

These results show that the most abundant proteid of the sunflower seed consists of a single globulin, and that the proteid precipitated by saturating with sodium chloride contains the same amount of nitrogen as the proteid soluble in a saturated solution of this salt. As Vines stated that the substance of the aleurone grains was soluble in a saturated salt solution after treatment with alcohol, while after treatment with ether it was insoluble therein, although soluble in ten per cent. salt solution, we thought that possibly by treating our meal with alcohol we might remove some substance, perhaps an acid soluble in alcohol, but insoluble in ether, which might be the cause of this peculiar behavior of the proteid. We accordingly extracted a quantity of sunflower meal with alcohol of 0.820 sp. gr., and in order to determine whether acid had been removed we attempted to titrate a portion of the extract with a one per cent. solution of potash. On adding the alkali a colored precipitate resulted, which rendered the indicator (*phenolphthalein*) useless. The attempt was then repeated, omitting the indicator. When the potash solution was added a bright chrome yellow color resulted which gradually increased with the formation of a precipitate as the quantity of the potash was increased. With a larger excess of potash the precipitate redissolved. This reaction we found to be due to helianthotannic acid.[1] The results of our investigation of this acid will be given in another paper.

Having now found a very delicate test for this acid, we applied it to our preparations of globulin and obtained a strong reaction in every case. It was therefore necessary to remove this acid from the meal before attempting to obtain the proteid and accordingly the extraction of the meal with alcohol was continued. It was, however, practically impossible to remove the acid so completely as to obtain no yellow reaction when the extract was treated with potash.

The meal which had been nearly freed from this acid was washed with ether and air-dried. 100 grams were extracted with ten per cent. sodium chloride brine and the filtered extract saturated with salt. An abundant precipitate separated, just as

[1] Ludwig and Kromayer, N. Br., 99, 1, 285.

with meal which had not been treated with alcohol. This was filtered off, dissolved in ten per cent. brine and again precipitated by saturation with salt. This precipitate was again dissolved in salt solution, filtered perfectly clear, and dialyzed. The globulin which was thus precipitated was filtered out, washed with water and alcohol and dried over sulphuric acid. This preparation, 10, weighed seven and four-tenths grams and had the following composition:

SUNFLOWER GLOBULIN, PREPARATION 10.

Carbon	51.27
Hydrogen	6.55
Nitrogen	18.21
Sulphur	0.78
Oxygen	23.25
	100.00
Ash	0.31

The saturated sodium chloride solutions filtered from the two precipitations of 10, were united and dialyzed until free from chloride; the resulting precipitate was filtered out and treated as 10 had been. Preparation 12 was thus obtained, which on analysis gave the following results:

SUNFLOWER GLOBULIN, PREPARATION 12.

Carbon	51.58
Hydrogen	6.55
Nitrogen	18.29
Sulphur	0.97
Oxygen	22.61
	100.00
Ash	0.29

As both the preceding preparations were found to contain detectable quantities of helianthotannic acid another attempt was made to prepare some meal which should be practically free from this acid.

One hundred grams of meal were therefore extracted in a Squibb's percolator with alcohol of 0.820 sp. gr., the whole being kept at 65° C. until 1500 cc. of extract were obtained.

The temperature was then raised to 75° and the extraction continued, about seven liters of alcohol being passed through the

meal. The last two liters were evaporated and left a residue weighing only 0.28 gram.

The meal residue was air-dried and extracted with ten per cent. sodium chloride solution. The extract was then filtered clear and saturated with ammonium sulphate, the precipitated proteid filtered out, dissolved in brine, the solution filtered perfectly clear and dialyzed.

The proteid was thus precipitated in large spheroids and was filtered out, washed with water and alcohol, dried over sulphuric acid and found to weigh 15.5 grams, preparation 13. This substance was freer from coloring matter than any before made, and had the following composition :

SUNFLOWER GLOBULIN, PREPARATION 13.

Carbon	51.54
Hydrogen	6.99
Nitrogen	18.58
Sulphur	1.00
Oxygen	21.71
	100.00
Ash	0.47

This preparation, which was very nearly white in color after drying, dissolved almost wholly in ten per cent. sodium chloride brine at 20°, giving a solution slightly tinged with greenish brown, which on dilution yielded an abundant precipitate that on warming, while suspended in the diluted solution, redissolved completely and again separated on cooling in spheroids, and on settling united with a coherent layer.

Solutions in ten per cent. sodium chloride brine behaved as follows :

When saturated with magnesium sulphate at 20° or sodium sulphate at 34°, the proteid was completely thrown out of the solution. When saturated with sodium chloride it was partly precipitated.

With mercuric chloride, picric acid, or tannic acid a heavy precipitate was produced.

With minute quantities of nitric, sulphuric, hydrochloric, or acetic acid the globulin was precipitated.

In pure water this preparation formed a plastic mass, but none dissolved.

In the water containing a minute quantity of acid it dissolved readily and completely.

With the xanthoproteic, Millon's, biuret and Adamkiewics' tests the usual preteid reactions were obtained.

When dissolved in ten per cent. sodium chloride solution and tested for heat coagulation point in the usual manner a turbidity formed at 90°, and a flocculent coagulum began to separate at 93°, increasing as the temperature was raised toward 100°. After heating sometime in a boiling water bath a considerable coagulum formed, yet a large proportion of the substance still remained in solution, as shown by the voluminous precipitate produced on adding acetic acid to the solution filtered from the coagulum.

In composition and reaction this preparation agrees with the globulin edestin, except that a part is precipitated by saturating its solutions in brine with sodium chloride. In composition the part precipitated by saturating with salt and that remaining in solution are alike. We have in another paper[1] pointed out that the globulin of the castor bean shows a similar behavior, and that the part precipitated by saturating with salt is a derivative of the part soluble in saturated salt solutions. We have further shown that the addition to a solution of edestin of a quantity of acetic acid too small to detect after mixing with the proteid, causes a precipitation of the edestin on saturating its solution with brine, and that under these conditions, the proteid otherwise behaves like the globulin from the castor bean and sunflower seed.

As helianthotannic acid contains about fifty-three per cent. of carbon, the presence of two per cent. of this acid in our preparation would but slightly raise the figures obtained for carbon and reduce those for nitrogen by about 0.35 per cent. The composition of the purer preparations which we have obtained differ from edestin to about this extent.

It is therefore our opinion that the sunflower seed contains as its principal proteid the globulin edestin, but that as obtained by extraction from the seed, this is mixed with helianthotannic acid, from which we have not succeeded in separating it completely.

Having thus found that a large part of this globulin is insolu-

[1] This Journal, 19, 482.

ble in saturated salt solutions under all the conditions of our tests, we were led to repeat Vines's experiments, but have been unable to confirm his observations, the aleurone grains appearing to be wholly unaffected by saturated salt solution after treatment of the seed with alcohol.

THE PROTEIDS OF THE COW PEA.[1]

(Vigna Catjang.)

By Thomas B. Osborne and George F. Campbell.

Received April 12, 1897.

THE proteids of this plant have never been, to our knowledge, the subject of study. Because of its great and increasing agricultural importance, and as a plant differing botanically from those included in our investigation of "legumin," the proteids of its seeds have much interest. The material examined was prepared by coarsely grinding the peas, separating the black seedcoats by a current of air, and then grinding the coarse meal to a fine flour. Two kilograms of this flour were treated with a quantity of ten per cent. sodium chloride solution, the extract was strained through fine bolting-cloth and allowed for three hours to deposit the greater part of the suspended starch. The extract was then run through a DeLaval centrifugal separator, whereby most of the remaining suspended starch and fiber was removed, and lastly was filtered perfectly clear by passing through a thick layer of filter paper pulp. The extract was saturated with ammonium sulphate, the precipitated proteids collected on a filter and dissolved in brine. The solution was filtered perfectly clear and dialyzed for four days.

The proteid, thus separated in the form of spheroids, was designated *A*, and the solution filtered therefrom was marked *B*. *A* was collected on several paper filters. One portion was washed very thoroughly with water and with alcohol and, dried over sulphuric acid, gave preparation 1, which weighed 29.7 grams. The rest of *A* was dissolved in one liter of five per cent. sodium chloride brine, and the solution filtered perfectly clear. On adding one liter of distilled water a large precipitate, *D*, separated, which was allowed to settle over night. The liquid, *C*,

[1] From the Report of the Connecticut Agricultural Experiment Station for 1896.

was then decanted from the proteid, *D*, which had formed a coherent deposit. *D* was dissolved in 150 cc. of ten per cent. sodium chloride brine, with which it readily yielded a clear solution, and water was added to make the volume one liter. A rapidly settling precipitate appeared that soon united to a coherent layer, from which the nearly clear liquid was decanted. After thoroughly washing this precipitate with water and with alcohol and drying it over sulphuric acid, there resulted 50.9 grams of preparation 2. The solution decanted from 2 was treated with 200 cc. of water, causing a precipitate which, washed and dried as before, gave 12.83 grams of preparation 3. Similarly the solution decanted from 3, when mixed with 200 cc. more water, gave six and two-tenths grams of preparation 4.

The solution decanted from 4 was dialyzed until chlorides were removed, which treatment precipitated all but a trace of the dissolved proteids. In this way four and four-tenths grams of preparation 5 were obtained.

The solution *C*, decanted from the precipitate *D*, as described on page 494, was diluted with 500 cc. of water. The proteid thus thrown down, after washing and drying, formed preparation 6, which weighed 16.9 grams. The filtrate from 6 was dialyzed for two days, and a deposit obtained weighing, when dry, 10.5 grams, 7. The filtrate from 7 was nearly free from proteid matter.

These preparations were analyzed, after drying at 110°, with the following results:

	1.	2.	3.	4.	5.	6.	7.
Carbon.....	52.45	52.69	52.63	52.56	52.52	52.59	52.27
Hydrogen...	6.92	6.77	6.90	6.98	7.04	7.08	6.97
Nitrogen....	17.16	17.18	17.50	17.18	17.27	17.24	16.69
Sulphur.....	0.40	0.57	0.52	0.62	0.53	0.56	0.50
Oxygen	23.07	22.77	22.45	22.66	22.64	22.53	23.57
	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Ash.....	0.32	0.64	0.47	0.50	0.42	0.45	0.16

It will be noticed that the first six preparations are nearly identical in composition. Of these, 1 represents the globulin precipitated by dialyzing the solution of the ammonium sulphate precipitate (which contained all the proteid matter extracted from the seed). 2, 3, 4, and 5 are four fractions of that portion of the substance (of which 1 is a part) that is most readily precipitated by dilution. 6, which also agrees with the foregoing,

is that part of the remainder thrown down by further dilution, the solution filtered from the precipitate, yielding 2, 3, 4 and 5. In composition, 7 differs from 1–6. Although the figures obtained for 1 closely agree with those for 2, 3, 4, and 5, the former has been shown to contain about ten per cent. of the globulin, 7, whose presence in this proportion affects but slightly the percentage composition of 1.

Remarking that 7 agrees in composition with the globulin phaseolin and that preparations 1–6 have a composition corresponding to a mixture of equal parts of phaseolin and legumin, we thought it important to determine whether or not this substance contained these two proteids. Phaseolin being much more readily soluble in dilute saline solutions than legumin, these globulins may be separated by fractional precipitation. Accordingly, twenty grams of 2 were treated with 500 cc. of five per cent. sodium chloride brine, and the solution was filtered off from the undissolved "albuminate." The filtrate, which measured 378 cc., was diluted with four times its volume of water, to produce a one per cent. brine, in which phaseolin dissolves freely and legumin somewhat sparingly. An abundant precipitate fell which was filtered out, washed, and dried, as usual, and formed preparation 8. The filtrate from 8 was saturated with ammonium sulphate, and the very small precipitate produced was dissolved in water and dialyzed. Only three-tenths gram of globulin was thus obtained, showing that very little of the proteid was soluble in one per cent. salt solution.

The residue which remained undissolved after treating 2 with five per cent. sodium chloride brine, was washed by decantation with salt solution, heated to about 50°, and the filtered washings dialyzed. Preparation 9 was thus precipitated.

The portion of 2 that remained undissolved by this treatment was thoroughly washed with water and alcohol and dried over sulphuric acid, making preparation 10. These were dried at 110° and analyzed with results that follow:

	8.	9.	10.
Carbon	52.74	52.70	52.87
Hydrogen	6.94	6.97	6.95
Nitrogen	17.22	17.21	17.29
Sulphur	0.40	0.42	0.49
Oxygen	22.70	22.70	22.40
	100.00	100.00	100.00
Ash	0.66	0.73	0.64

It is evident that no fractional separation was accomplished by this treatment, and that the most abundant globulin of the cow pea must be regarded as a distinct proteid, differing from any heretofore described. For this proteid we propose the name *vignin*.

Its composition as represented by an average of the foregoing figures is here stated.

VIGNIN.

Carbon	52.64
Hydrogen	6.95
Nitrogen	17.25
Sulphur	0.50
Oxygen	22.66
	100.00

The reactions of vignin are as follows: In cold or warm water, when free from soluble salts, it dissolves to a considerable extent, the solutions resulting being precipitated by adding a very little salt. The precipitate so separated dissolves completely on adding more salt.

It is readily soluble in sodium chloride solutions, containing upwards of five per cent. of salt, from which solutions it is promptly precipitated by dilution. In two and one-half per cent. salt solutions relatively little, and in one per cent. very little, dissolves. It is readily and completely soluble in dilute acids and alkalies in absence of salts. Solutions in very dilute nitric or hydrochloric acid are precipitated by salt or by an excess of these acids. In dilute sulphuric acid it is much less readily soluble than in the acids just named, and is not precipitated by an excess of sulphuric acid and but slightly on adding salt. Solutions in very dilute acetic acid are not precipitated by an excess of this acid but give heavy precipitates on adding sodium chloride. Dissolved in one-half per cent. sodium carbonate solution, the proteid is precipitated by neutralization. The separated proteid at once dissolved on adding salt. Dissolved in ten per cent. sodium chloride brine, this globulin behaves as follows:

By hydrochloric acid it is not precipitated until a relatively considerable quantity of the acid is added. By this acid the globulin is less readily thrown down than legumin but more readily than phaseolin.

The same is true of acetic acid, of which a somewhat large quantity is required to cause a precipitate. Phaseolin is not precipitated at all under these conditions.

By diluting the salt solutions of vignin it is apparently more readily precipitated than legumin, and far more readily than phaseolin.

Saturation of solutions of vignin with sodium chloride or magnesium sulphate gives no precipitate, but saturation with sodium sulphate at 34° causes nearly complete precipitation of the proteid.

Mercuric chloride gives no precipitate, tannic acid and picric acid make heavy precipitates in its solutions.

With Adamkiewics', Millon's, the biuret and xanthoproteic tests the usual proteid reactions are obtained.

Strong solutions of this globulin dissolved in ten per cent. sodium chloride become turbid when heated to 98°, and after continued heating set to a jelly.

Besides vignin the cow pea contains a small quantity of proteid matter represented by preparation 7, which in composition, as well as reactions, agrees very closely with phaseolin. Phaseolin differs from all other plant globulins, which we have thus far observed, in not being precipitated from its solution in ten per cent. sodium chloride brine by a large quantity of acetic acid. In this respect 7 behaves like phaseolin.

The composition of phaseolin as obtained from different seeds is shown by the following statement:

PHASEOLIN.

	Cow pea.	Kidney bean.	Adzuki bean.
Carbon	52.27	52.58	52.56
Hydrogen	6.97	6.84	6.97
Nitrogen	16.69	16.47	16.45
Sulphur	0.50	0.56	0.57
Oxygen	23.57	23.55	23.45
	100.00	100.00	100.00

The solution *B*, filtered from *A*, as described on page 494, was saturated with ammonium sulphate and the small precipitate produced was dissolved in a little dilute salt solution, filtered perfectly clear, and dialyzed for two weeks. A precipitate resulted which was filtered out, washed with water and alcohol,

and dried over sulphuric acid. This preparation, 11, weighed three and two-tenths grams. The filtrate from 11 was dialyzed ten days longer, but as nothing separated the solution was dialyzed against alcohol. A small precipitate, 12, appeared, which weighed 3.75 grams.

These preparations, after drying at 110°, were analyzed with the following results:

	11.	12.
Carbon	53.13	53.36
Hydrogen	7.09	7.05
Nitrogen	16.51	16.21
Sulphur	1.09	1.13
Oxygen	22.18	22.25
	100.00	100.00
Ash	0.65	0.81

It will be noticed that these two preparations agree quite closely in composition. Although 11 was precipitated by dialysis in water and is unquestionably a globulin, while 12 could not be precipitated by even prolonged dialysis, nevertheless it is our opinion that these are one and the same proteid. In a subsequent paper we hope to point out the relations of this globulin to the proteids obtained similarly from other leguminous seeds.

CONCLUSION.

1. The chief proteid of the cow pea is a globulin, much resembling the legumin of the pea and vetch, but essentially different in composition and properties, for which we propose the name *vignin*. Its composition, as found by the average of closely agreeing analyses of nine fractional precipitates, is as follows:

VIGNIN.

Carbon	52.64
Hydrogen	6.95
Nitrogen	17.25
Sulphur	0.50
Oxygen	22.66
	100.00

2. Besides vignin, the cow pea contains a globulin which has the composition and, so far as could be determined, the properties of phaseolin, which we have found in the kidney bean

(*Phaseolus vulgaris*), and the adzuki bean (*Phaseolus radiatus*).

3. The cow pea contains a third globulin, extremely soluble in very dilute salt solutions, which could be precipitated but partially by dialysis in water and completely only in the coagulated form by dialysis in alcohol. This substance closely resembles, in properties and composition, bodies obtained from several other leguminous seeds. Its composition, as found by analysis of two precipitates, one obtained by dialysis in water and the other by further dialysis in alcohol, is as follows:

Carbon	53.25
Hydrogen	7.07
Nitrogen	16.36
Sulphur	1.11
Oxygen	22.21
	100.00

ON THE MANUFACTURE OF DYNAMITE.

By G. E. Barton.

Received April 22, 1897.

UNDER the general term dynamite are included two classes of mixtures. The first of these consists of nitroglycerol, held by an absorbing medium, such as woodpulp or kieselguhr, together with various other bodies used as fillers, the principal one being sodium nitrate; this latter body also adds to the strength of the dynamite when woodpulp is the absorbent used by furnishing oxygen for the combustion. The second class, more properly called gelatin dynamites, consists of nitroglycerol gelatinized by means of nitrated cellulose, much the same substances being present as fillers, but considerable less absorbing material in proportion to the amount of nitroglycerol. It is to the former class of mixtures that this paper relates, although most of it is equally applicable to the latter. Most of the dynamite used in this country is of the first class, as it is less costly and equally serviceable, except in wet holes.

The choosing of a site for a dynamite factory is a matter into which many factors enter. It is much more convenient, cheaper, and safer to have the part of the plant used in making the nitroglycerol on a hillside, as it is then possible to allow that liquid to flow from building to building in pipe lines. Authorities usually hold that the soil should not be sandy on account of the

danger from sand blowing into the buildings and finally giving rise to friction in some part of the apparatus. The danger from this source is very small, as can be seen from the fact that one factory (the only one known to the author on such soil) has been located for about fifteen years on a sandy hillside without having an accident which could be traced to this cause. The only precautions taken are to carefully clean everything after each day's work, which must be done anyway, and the sweeping out of the sand occasionally as it accumulates before the doors. The sandy soil probably plays a very important part in another direction, for though the most violent thunder storms sweep over the region during many months of the year, there has never been a building struck by lightning. No lightning protectors whatever are used. The explanation of this fact seems to be that the light buildings, with their very light foundations, are much poorer conductors of electricity than the surrounding trees, the roots of which extend into the moist earth below. Another advantage is that the shock of the most violent explosion is not conducted to great distances, as is the case in other soils. In the factory before mentioned a catch box for the nitroglycerol, carried away in the wash waters, in which about one hundred and fifty pounds of that explosive had accumulated (contrary to orders) exploded. It was situated not more than one hundred feet from the storehouse, in which was considerable nitroglycerol at the time, but this did not explode, although the flying débris damaged the building badly.

The manufacture of nitroglycerol is the most important point to be considered in connection with the manufacture of dynamite and the different parts of the process will be taken up in the order in which they occur. The nitration is best conducted in a nitrator of cylindrical shape and made of lead with a bottom sloping slightly toward the point at which the faucet for drawing off the mixture of nitroglycerol and acids is placed. The top is made of lead and has several panes of glass set in it, through which the workman watches the operation. From the center a ventilator of lead passes up through the roof. Two thermometers are fixed, one with the bulb near the surface of the liquid, and the other with it in the lower strata. These are in front of the operator at work, and guide him. The glycerol valve is

controlled with the left hand, and the compressed air for agitating with the right.

The method of mixing by compressed air is beyond doubt the safest, as it gives a more thorough mixing, allows more than one cooling coil to be used, and is less liable to get out of order. A drawback, common to all mechanical agitators, is the unreliability of any piece of machinery exposed to the acid fumes which are always present in and near the nitrating house. The air after leaving the compressor should be passed through an iron drum containing sulphuric acid, and arranged in the same manner as the ordinary bottle for washing gases in the laboratory, in order that any moisture it may contain may be removed.

Everything being in order and tested as far as possible, the operator sets the lead trough, which connects the discharging faucet with the separator, or drowning tank, as desired, to connect with the drowning tank, starts the water running into the tank so as to give some circulation and consequent stirring there in case it becomes necessary to drown the charge and allows the mixed acids to run into the nitrator from a small tank, into which just enough for one charge has previously been forced from the acid store tank outside the danger area by means of a montejus and compressed air. The cooling coils furnish enough water to keep up a circulation in the drowning tank when allowed to discharge into it. About 1,500 pounds of mixed acid is found to be an economical charge, and to this should be added from 210 to 230 pounds of glycerol, according to the conditions. It is economical to increase the glycerol used, even though the percentage yield decreases, as long as a pound of glycerol adds a pound to the yield of nitroglycerol. The acids used should have the following composition: Sulphuric acid, 61.9 per cent.; nitric acid, 34.5 per cent.; oxides of nitrogen, calculated as nitrogen trioxide, 0.7 per cent., with not more than a trace of chlorine and iron. The glycerol must have approximately the following composition, when examined by the method given in this Journal, **17**, 277: Ash, trace; carbonaceous residue, 0.012 per cent.; sodium chloride, 0.002 per cent.; total acid equivalent, 0.05347; permanent specific gravity, 1.2653; specific gravity, 1.2634; higher fatty acids, none; reaction, neutral. During the process of nitration the temperature should not

be allowed to go above 88° F. The yield by this method is greater the shorter the time employed in the process, so that in the winter, when a run can be made in one-half an hour or less, fifteen or twenty pounds more of glycerol can be used without decreasing the percentage yield. This is partly due to the fact that the shorter the time the less nitric acid is blown out of the mixture in agitating. No economical method has been devised for recovering the acid thus lost or for reducing the temperature during the summer to the point easily obtainable in this climate during the winter months.

The nitration being finished, the trough is placed so that the mixture can flow into the separating boxes, which transfer ought not to take over three minutes. It is usually held that the separators should be in another building, but many factories in this country have them in the same one with the nitrator. By this method only one drowning tank is required, while if the buildings are far enough apart for one to escape injury from an explosion in the other, two must be provided. It also avoids the trouble which would otherwise be caused by passing the mixture of acids and nitroglycerol through a long trough.

It is almost impossible to avoid some of the mixture remaining in the hollows of the trough, and this in a few hours, especially during the summer, becomes dangerous.

While an explosion would not necessarily be communicated to either building, it must at least injure the line and delay work. No way would be at hand of disposing of the run then in the nitrator, except by drowning, with the consequent loss of the spent acids. The disadvantage is that an explosion destroys more apparatus and material. The danger in a building containing the separators and nitrator is not increased to any extent, as both operations, when properly conducted, give ample warning before an explosion. The building must, of course, be arranged with several exits.

Two men working with two separators and a nitrator, can easily dispose of twelve runs in a working day of ten hours, which means 5,500 pounds of nitroglycerol. If it is necessary to make more, such a plant can be forced to fourteen or fifteen runs a day, under favorable conditions, by working a little overtime. If still more nitroglycerol be required, it is best to build another

similar plant, far enough removed from the first to avoid danger. This latter plan also has the advantage that a single explosion does not entirely stop the production, which is a thing greatly to be desired, as anyone familiar with the demands of trade for the rapid filling of orders will appreciate.

The separators should be cylindrical lead boxes, containing both cooling coils and an arrangement for agitating with air, the latter for use only in emergencies. The bottom is made slightly sloping towards the point where the cock for drawing off both acids and nitroglycerol is placed. The top should be covered with lead, and have glass windows for viewing the fumes given off during the operation. It is necessary to have a narrow pane of glass in the side next to the cock, by means of which the operator is enabled to see when all the acids have been drawn off, and interrupt the flow before any of the nitroglycerol goes into the second separator. The spent acids having been disposed of, a short lead trough is then set so that the nitroglycerol can flow in a thin sheet into a small tank half filled with water, and connected with the wash-house. A stream of water, sufficient to keep the liquid in this tank at a constant level, is turned on at the same time. The nitroglycerol flows from here into the wash-tank in the wash-house, the stream of water being kept up till the nitroglycerol is drowned, and a sufficient quantity of water passed through the pipe line to clean out the last traces.

The wash-tanks should be made of wood, and lined with lead. The agitation is best done with compressed air. Two faucets are set in each of these tanks, one at the bottom for drawing off the nitroglycerol at the end of the washing, and one above the surface of the explosive through which the wash-waters escape. An alkaline wash must always be used at least once, and the nitroglycerol tested with litmus paper before it is allowed to go to the storehouse. The disposal of the wash-waters is a problem which every manager must solve for himself according to conditions at hand, but it will effect quite a saving if they are allowed to flow through a series of compartments in which the little nitroglycerol left in them has a chance to settle out. The nitroglycerol should be removed from these compartments at least once a week. The lead lining to the wash-tanks should be insisted upon, as otherwise the fibers of the wood are opened by

the action of the acid and alkali, and become saturated with nitroglycerol, making the repairing or destruction of the tanks dangerous. The author has traced at least one explosion to this cause.

From the wash-house, the nitroglycerol may be allowed to flow at once into the storage tanks, which are in a separate building. In this country, where there is no limit placed on the amount of nitroglycerol which may be stored, or the length of time it may be kept, no filtering or drying process is necessary. There should be several tanks in the storehouse, and the nitroglycerol should stand twenty-four hours before being mixed with the absorbent, thus giving time for the small amount of moisture which it still contains to become thoroughly separated. Each tank should be tested with litmus paper every day, and if any signs of acidity are developed, the nitroglycerol must at once be destroyed or rewashed. The pipe line from the wash-house should be so arranged that it may be broken when not in actual use, thus preventing the communication of an explosion from one building to the other. It should also be carefully guarded against freezing in winter, and becoming overheated in summer.

The spent acid, which we lost track of as it left the first separators, flows in the usual form of trough to the second separators, where the process is continued for at least three, preferably five days. These separators are best oblong lead boxes, having a capacity sufficient to accommodate the spent acid from three runs, and are furnished with cooling coils and air agitators, the last for use only in emergencies. The top of these boxes should have the shape of three quadrangular pyramids, set side by side. These pyramids terminate in glass cylinders about twelve inches in height and four in diameter, which have a glass plug ground into them about six inches from the bottom. The tops of these cylinders are covered with ground glass plates in order that the air may be excluded from the surface of the liquid as much as possible. The top plates having been removed, the spent acid is allowed to flow into the box until it is filled nearly to the top of the glass cylinders. The nitroglycerol finds its way to the surface of the liquid, and when it has accumulated to the depth of an inch or so, the lower level is brought opposite the plug in the side of the cylinder by drawing out a little of the spent acids,

the plug is removed and the nitroglycerol allowed to flow out and into a lead saucer just below, from which it passes into a dish set to catch it under the spout of the saucer. It must be at once emptied into a pail and carried to the wash-house to be washed, or else drowned in a small tank provided in the separating house. The latter plan is preferable, as the danger of an explosion from the shock of dropping a pail is much less after the nitroglycerol has been thoroughly washed.

How to dispose of the spent acids from the nitroglycerol manufacture, which contain from seventy to seventy-four per cent. of sulphuric acid and from seven to twelve per cent. of nitric acid, is a question which probably has caused managers of dynamite factories more thought than almost any other problem connected with the business. It can hardly be said to be solved satisfactorily yet, but in this country the weight of opinion is in favor of regaining the acid. For this purpose it is run at once from the second separators into a montejus and blown into a tank situated near the top of a Glover tower from which it can be run directly into the tower without further precautions. The nitric acid is condensed in any of the usual ways, while the sulphuric acid runs into a tank at the foot of the tower. The sulphuric acid comes out at 58° B, or over, in a well-managed tower, and the nitric acid can be kept above 36° all the time, usually near 38° B. Care must be taken to arrange the pipe leading from the montejus at the separating house to the tank at the Glover tower, so that it will drain itself thoroughly, as otherwise a sediment, composed mostly of lead sulphate, will settle out, together with any small amount of nitroglycerol the acids may still contain, and finally cause an explosion.

A few general rules to be observed in the manufacture of nitroglycerol may be mentioned here:

Nitroglycerol should never be carried in pails when it is possible to avoid it.

All receptacles which contain the nitroglycerol and acids at the same time should be covered so that neither water nor other foreign substance may accidentally come in contact with them. The writer has seen heating sufficient to necessitate the drowning of the contents of one of the second separators, caused by a small piece of wood falling into the mixture.

All receptacles for nitroglycerol, or nitroglycerol and acids together, should be set on a slight incline, so that they may be drained thoroughly on emptying them.

No more nitroglycerol should be made in one plant in a day than can be handled by two men working in a combined nitrating and separating house as described above. If more is to be handled, even occasionally, it will pay to have another plant entirely separate. The second plant is very valuable in case of emergencies, either due to explosions or the necessity for repairs to the apparatus.

The quality of the mixed acids and glycerol used must be given careful attention, and both should be bought under specifications embodying the analytical method. The method given by Lunge for the analysis of the acids is best for this purpose, while that given in this Journal, 17, 277, was devised by the author especially for such use in the case of glycerol. The strict enforcement of such specifications has led to the saving of at least $5,000 in a single year in the case of a factory turning out 9,000 or 10,000 pounds of dynamite per day.

The mixing of the nitroglycerol with the absorbent may be conducted in a building on the same level as the storehouse for that explosive. The best form of mixing machine now in use, is practically the well-known wheel-mill of the powder manufacturer. It consists of a circular wooden trough capable of containing a mixing of 500 pounds of dynamite at a time. In this trough, two large wooden wheels run, each followed by a brass plough which has a strip of rubber fastened at the lower edge, thus cleaning the trough without requiring an actual contact. Such an apparatus requires less time for mixing than the old method of using rakes, and is more thorough. The only danger is from nails, or other foreign substances getting into the trough, and to avoid this the absorbent should always be sifted. This is done best before the different ingredients are put together. The nitroglycerol should be brought from the storehouse in a small lead-lined tank, placed on wheels, and arranged as a push-cart. This is wheeled upon scales set in the floor of the storehouse, counterbalanced, and the required amount of nitroglycerol for one mix, run into it through an opening in the top. It is then wheeled along a platform to the mix-house.

This platform should have guides to prevent the cart from running off. At the mix-house, the nitroglycerol flows from the tank into the mixing trough through a piece of rubber hose attached to a small spigot in the bottom of the tank. The end of this hose is securely fastened to the top of the tank during the weighing and transportation of the nitroglycerol. This plan for transporting the explosive is superior to any of the old methods using pails, since they, even at the best, require some handling, with the consequent danger of dropping. The danger of spilling is also reduced to a minimum by this method.

The dynamite next passes to the pack-houses, where it is packed in paper cartridges of almost any desired size. Many packing machines are in use, but the old method of filling the cartridge by placing the open end over the tube of a tin funnel, and pressing the dynamite into place with a wooden packing stick, is preferable on the score of safety. From the pack-house the cartridges pass to the casing house, which process presents no particular points of difficulty, and from there to the magazines, or cars, as the case may be.

We now come to the part of the manufacture which is carried on outside the danger limits, and which includes the regaining of the spent acids already described. The arrangement of this part of the plant is solely with regard to convenience, and offers no particular difficulties due to the nature of the work with the possible exception of the storehouse for the sodium nitrate, which should be at a reasonable distance from other buildings, and upon which no water should be thrown in case of fire. The sodium nitrate is best ground and dried in a steam-jacketed pan, in which heavy iron rollers run, as in the wheel-mill before mentioned. Steam-jacketed pans are also provided for the wood-pulp, kieselguhr, etc.

The paper cartridges were formerly made by hand, and a large factory required a considerable number of girls for this purpose; but at least one machine is known to the writer, which does satisfactory work for a considerable range of diameters and lengths by a very simple adjustment.

Besides the examination of the mixed acids and glycerol above mentioned, the chemist should make frequent determinations of the nitroglycerol in the finished product, as a check on the mix-

ing and weighing. The spent and regained acids may be analyzed as a check on the working of the regaining plant, while valuable information is often brought out by an examination of the various absorbents.

MILLVILLE, N. J., APRIL, 1897.

PROTEID OF THE WHITE PODDED ADZUKI BEAN.[1]

(*Phaseolus Radiatus*).

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

Received April 12, 1897.

THIS is a small red bean cultivated in Japan. The seeds used in this investigation were grown in Kansas and sent to us by Professor C. C. Georgeson.

As our object was to discover the nature of the globulin forming the chief proteid constituent of various leguminous seeds, no attempt was made to determine the total amount of proteid contained in this seed, nor to study the other proteid substances occurring in small quantity.

It was impossible, by any means at our command, to remove the closely adhering red seed-coat, but as it was found that this yielded but little coloring matter to salt solution the entire seed was ground until all passed a sieve of fine bolting cloth.

Two kilograms of this meal were treated with eight liters of ten per cent. sodium chloride solution, and after stirring some time the mixture was strained on fine bolting cloth.

After standing long enough to deposit most of the suspended starch the extract was filtered quite clear and saturated with ammonium sulphate. The precipitate so produced was filtered out, suspended in water, and in order to remove the adherent ammonium sulphate, which prevented solution of the proteid in a sufficiently small volume of water, the mixture was dialyzed over night. The proteid was thus completely dissolved. The solution was filtered perfectly clear and again dialyzed four days. A large precipitate of globulin resulted, which was filtered out and a portion collected on a separate paper and washed thoroughly with water and alcohol and dried over sulphuric acid. This was found to weigh 13.21 grams and formed preparation 1.

[1] From the Report of the Connecticut Agricultural Experiment Station for 1896.

The remainder of the globulin was suspended in 850 cc. of water and 150 cc. of ten per cent. salt solution added, which yielded a nearly clear solution, showing the globulin to be readily soluble in one and a half per cent. brine. The solution was filtered clear and the filter washed with 100 cc. of one and a half per cent. salt solution. The filtered liquid, measuring 1100 cc., was treated with 500 cc. of water, thereby throwing down a large rapidly settling precipitate, which, after decanting the fluid, was dissolved in ten per cent. salt solution, filtered perfectly clear and dialyzed for three days. The globulin was thus nearly completely precipitated, for further dialysis of the filtered solution caused separation of very little more. Under the microscope the globulin appeared as well developed spheroids. After filtering this precipitete was washed with water and with alcohol and dried over sulphuric acid. This formed preparation 2, weighing 41.5 grams.

The solution decanted from the first precipitation of 2, caused by the addition of 500 cc. of water as described above, was further diluted with 500 cc. of water, which caused an abundant separation of proteid. After settling over night the clear solution was decanted from the precipitate and the latter washed thoroughly with water and with alcohol and dried over sulphuric acid, giving preparation 3, weighing 31.34 grams.

The solution decanted from 3 was cooled over night in an ice chest to 9°, which caused a further separation of proteid in large spheroids. This was filtered off, washed with water and alcohol, dried over sulphuric acid and found to weigh 6.18 grams, preparation 4.

The filtrate from 4 was dialyzed for twenty-four hours and filtered from an abundant precipitate. The latter was washed with water and alcohol, and after drying weighed 12.0 grams, preparation 5. The filtrate from 5 gave only a trace of precipitate on further dialysis.

The preparations were dried to constant weight at 110° and analyzed. The following figures show that fractional precipitation had caused no separation and that the globulin is identical in composition with phaseolin as obtained from the white bean, *Phaseolus vulgaris*.[1]

[1] This Journal, 16, 633.

PHASEOLIN.

Adzuki Bean.

	1	2	3	4	5
Carbon	52.31	52.56	52.74	52.74	52.44
Hydrogen	7.03	6.98	6.94	6.97	6.91
Nitrogen	16.43	16.41	16.34	16.62	16.47
Sulphur	0.57	0.62	0.56	0.49	0.61
Oxygen	23.66	23.43	23.42	23.18	23.57
	100.00	100.00	100.00	100.00	100.00
Ash	0.16	0.73	0.32	0.90	0.05

Average Adzuki bean.	Average White bean.
52.56	52.58
6.97	6.84
16.45	16.47
0.57	0.56
23.45	23.55
100.00	100.00

The reactions of phaseolin, whether obtained from the common white bean or the adzuki, are as follows:

In cold and warm water it is entirely insoluble.

In sodium chloride solutions it is readily soluble, one per cent. brine dissolving large quantities of phaseolin.

In dilute acids and alkalies it is readily soluble; the solutions are precipitated by neutralization.

Solutions in dilute nitric or acetic acid are not precipitated even by large excess of acid.

Dissolved in one-fourth per cent. sodium carbonate solution, phaseolin is precipitated by neutralization, the precipitate being wholly dissolved on adding sodium chloride.

Phaseolin dissolved in ten per cent. sodium chloride brine reacts as follows: It is *not* precipitated even by large excess of hydrochloric, nitric, sulphuric or acetic acids. By a large quantity of trichloracetic acid a precipitate is produced. By dilution with a sufficient quantity of water a precipitate results. Saturation with sodium chloride or magnesium sulphate does not precipitate the phaseolin, but saturation with ammonium sulphate completely throws it out of solution, while saturation with sodium sulphate at 34° precipitates it mostly, but not entirely. Potassium ferrocyanide together with a large excess of acetic acid gives a precipitate.

With mercuric chloride dissolved in ten per cent. salt solution, no precipitate is produced. With tannin large precipitates result. With picric acid dissolved in ten per cent. salt solution, no precipitate forms until a considerable quantity of the acid is added, and the precipitate thus produced dissolves on adding salt solution.

Phaseolin gives with the biuret, Adamkiewics', Millon', sand xanthoproteic tests, the usual proteid reactions.

When solutions in ten per cent. sodium chloride brine are heated, turbidity occurs between 90° and 95° and a small flocculent coagulum separates at 97° to 98°; even prolonged heating in the boiling water-bath coagulates but a small part of this globulin.

The solution, filtered from globulin which had separated on dialyzing the solution of the proteids precipitated by saturating the seed extract with ammonium sulphate, was again saturated with this salt, and the precipitate thereby produced was dissolved in a small volume of water, and after filtering clear, the solution was dialyzed for six days. Three grams of a dark-colored globulin separated which seemed much contaminated with coloring matter. This was filtered out and the clear filtrate dialyzed five days longer, but no precipitate was obtained. The solution was then dialyzed into alcohol and the precipitate which resulted after drying weighed eight and a half grams. The filtrate contained but a trace of proteid. This substance was ground to a powder, thoroughly exhausted with water, washed with alcohol, dried and found to weigh 7.05 grams. Dried at 110° this preparation, 6, had the following composition, which it will be noticed is similar to that of preparations obtained in a like manner from the pea, vetch and cow pea.

Adzuki bean.	Pea.		Vetch.	Cow pea.	
6	5	8	20	8	9
53.97	53.33	53.54	53.55	53.13	53.36
7.01	6.98	6.99	6.70	7.09	7.05
16.31	16.14	16.69	16 46	16.51	16.21
0.88	1.00	1.01	1.02	1.09	1.13
21.83	22.55	21.77	22.27	22.18	22.25
100.00	100.00	100.00	100.00	100.00	100.00

Preparation 8 from the cow pea was obtained by prolonged dialysis in water, and accordingly must be regarded as a very

soluble globulin. Since preparations agreeing well in composition and general properties with it have been similarly obtained from other leguminous seeds by dialysis, it is our opinion that the above analyses represent a distinct globulin which can be only in part removed from its solutions by dialysis in water, but is wholly separated, in a coagulated form, by dialysis in alcohol. This globulin is at present being further investigated, and it is our intention to offer more respecting its properties in a subsequent paper.

NOTE.

The Electrolytic Determination of Cadmium.[1]—In preparing this paper we overlooked the paragraph on cadmium in Dr. Warwick's article, "Die Elektrolyse von Metall-Formiaten."[2] We are under obligations to Prof. Edgar F. Smith for calling attention to this oversight. S. AVERY and BENTON DALES.

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[1] This Journal, 19, 379.

[2] *Zischr. anorg. Chem.*, 1, 291.

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